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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A report on the progress of the research program of the US Army Biomedical Laboratory on Medical Defense Against Chemical Warfare Agents (W) for fiscal year 1980 is presented. Abstracts of the individual investigations are included on the DD Form 1498 introducing each work unit report.

FOREWORD

This FY 1980 Annual Progress Report is a general review of US Army Biomedical Laboratory, Aberdeen Proving Ground, MD conducted on Medical Defense Against Chemical Warfare Agents under projects 3M162734AH26, 3S162772A875 and 3M161102BS10.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978). The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

AUTHOR INDEX

USABML Annual Progress Report - FY 1980

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Executive Summary (Program Overview) (U)

The US Army Biomedical Laboratory (USABML) conducts research, development, test, and evaluation as it relates to medical defense against chemical warfare (CW). This mission includes fundamental research on mechanisms of action of CW agents and antidotes in order to establish a data base from which to devise improved prevention and treatment of casualties, and the development and evaluation of drugs and other methods for the prevention, resuscitation, pretreatment and management of chemical casualties.

The status of the USABML was changed from a subelement of DARCOM's Chemical Systems Laboratory to that of an independent laboratory under USAMRDC on 30 June 1979. Formation of this new laboratory required not only the establishment of a research and development program, but also the entire infrastructure to conduct this program.

Efforts to reorganize the laboratory functionally and programatically were begun in late October 1979. The ensuing new proposed provisional TDA was approved by HQ, USAMRDC in January 1980.

Although FY79 was the year of our conception as an independent laboratory under USAMRDC, FY80 was in essence the year of our birth, the year we wrote or rewrote all of our research plans and protocols. We are very proud to report progress in nine of these work units for our FY80 Annual Progress Report. Five work units are in project 3M162734AH26 "Medical Defense Against Chemical Agents," and two each are in projects 3S162772A875 "Medical Systems in NonConventional Environments," and 3M161102BS10 "Research on Military Disease, Injury and Health Hazards."

In general, research on two of these work units (024) "Efficacy of Centrally and Peripherally Active-Pretreatment and Treatment Compounds Against Nerve Agent Intoxication" and (381) "Mechanism of Action of Anticholinesterases and Anticholinesterase Antidotes" have been on-going for over 30 years with varying degrees of emphasis and varying approaches. New research plans were written for both of these work units in FY80.

One work unit (383) "Neurotransmitter Systems Interaction Effects of Anticholinesterases and Treatment Compounds" commenced in 1977. Two others (201) "Behavioral Toxicology of Nerve Agents and Treatment with Prophylactic and Therapeutic Compounds" and (202) "Physiological Consequences of Nerve Agent Exposure" were initiated in 1978.

Work unit (025) "Comparison of 4-DMAP, Sodium Nitrite, Amyl Nitrite and Sodium Thiosulfate; Efficacy of Treatment on Acute Cyanide Poisoning" began in April 1979.

The remaining three work units (021) "Use of Invertebrates as Model Systems for Investigating Effects of CW Agents and Treatment Compounds on Single Cells and Ganglia," (026) "Efficacy of Organophosphinates as Prophylactic Agents in Nerve Gas Intoxication," and (030) "Analyses for Potential Toxic Material(s) in Aged Atropen Injector" were introduced in 1980.

3M162734AH26 "Medical Defense Against Chemical Agents"

AH26AH021 "Use of Invertebrates as Model Systems for Investigating Effects of CW Agents and Treatment Compounds on Single Cells and Ganglia"

In order to develop an effective treatment or pretreatment for the soldier against nerve agent poisoning we need to know, amongst other things, the direct neuronal effects of both the agents and the treatments (or pretreatments). Sophisticated electrophysiological equipment designed for intracellular recording, for application of voltage and current, along with micropressure ejection apparatus for extracellular drug application and iontophoresis had to be acquired, and in many instances modified, before any data could be generated. We now have baseline data for Soman effects on three elementary acetylcholine (ACh) responses. As the restrictions for vertebrate use in research increases, use of this mollusc model in studying molecular mechanisms becomes more and more valuable.

AH26AC024 (U) "Efficacy of Centrally and Peripherally Active - Pretreatment and Treatment Compounds Against Nerve Agent Intoxication"

In this reporting period we were successful in developing two pretreatment mixtures for rats that were equally effective against Soman lethality. However, one of these mixtures containing physostigmine, atropine and mecamylamine was markedly superior to the other mixture in abolishing Soman-induced physical and mental debilitation. The other mixture contained pyridostigmine in lieu of physostigmine. Efforts in this area of our research are presently directed toward the idea that drug formulations for rats (and monkeys) must be based on criteria that are proposed for use in man, that is, behavioral titration versus survival titration (which obviously cannot be used in man). This means that responses other than survival must be used in selection of doses in experimental animals as well as in man, such as physical incapacitation, performance decrement and changes in behavioral stresses.

AH26AC025 (U) "Comparison of 4-DMAP, Sodium Nitrite, Amyl Nitrite and Sodium Thiosulfate: Efficacy of Treatment in Acute Cyanide Poisoning"

The lethal dose response curve to cyanide is being established. The effective dose of both sodium nitrite and 4-DMAP which produced the 30-40% methemoglobin level necessary for animal survival has been determined.

There are presently insufficient data on acute toxicity, chronic toxicity and carcinogenic properties of 4-DMAP. At present 4-DMAP is only manufactured in the IV formulation. Stability studies are needed for the IM formulation. It is known that 4-DMAP reacts with metals and certain kinds of rubber. Studies also should be performed with injectable container materials.

AH26AC026 "Efficacy of Organophosphinates as Prophylactic Agents in Nerve Gas Intoxication"

Having studied the hydrolytic stability, cholinesterase inhibition parameters and the responsiveness of the inhibited enzyme to oximes of ten phosphinate esters (from the twenty-six synthesized) two were examined further. Toxicological studies in mice have been completed with p nitrophenyl dimethyl phosphinate and p nitrophenyl methyl (phenyl) phosphinate. Not only are these two phosphinate esters less toxic than the carbamate pyridostigmine, but these preliminary studies suggest that they may be superior prophylactically.

Results so far in these studies are redirecting our concepts on the mechanisms of both carbamate and phosphinate prophylaxis.

AH26CA030 (U) "Analysis for Potential Toxic Material(s) in Aged Atropen Injector"

This study was the result of a directed action of the Commander of USAMRDC Walter Reed Institute of Research (WRAIR) completed the cytotoxicity studies which correlate with the mouse toxicities at USABML. Quantitative methods for the analysis of atropine, phenol and heavy metals have also been developed.

Zinc has been found to be the toxic material, originating in the rubber of the cartridge containing the active ingredients.

Project No. 3S162772A875: Medical Systems in Non-Conventional Environment

A875BA201 (U) Behavioral Toxicology of Nerve Agents and Treatment with Prophylactic and Therapeutic Compounds

Thirteen separate research protocols were active and productive in this work unit for FY80. Four research presentations were prepared and abstracts were submitted for publication. Three tests which reflect motor and behavioral incapacitation following sublethal exposure to Soman have been developed and validated. A therapeutic mixture reversing lethality and to an extent, motor incapacitation, has been tested. Also a prophylactic mixture reversing all effects has been tested. 2-PAM dose-response studies for behavioral efforts have been initiated. Dose-response studies of anticholinergic drugs on nonhuman primate learning and memory are on-going. Studies on the interaction between morphine, anticholinergics, anticholinesterase, and stress on pain preception are continuing.

A875202 (U) Physiological Consequences of Nerve Agent Exposure

Both the central and peripheral mechanisms of action of Soman on respiratory arrest have been the focus of this research during FY80. Differentiation of these mechanisms showed the cause of respiratory arrest was a loss of central drive due to a loss of syncnronized firing of respiratory-related neurons in the brain stem. Hence the need for centrally-active treatment compounds. We now have a model developed for testing the efficacy of future P&T compounds.

The preliminary results on the effects of an acute, sublethal exposure to Soman on sleep-wake cycles and arousal threshold suggest a need for long-term treatment of individuals following a single exposure to nerve agents.

Project No. 3M161102BS10: Research on Military Disease, Injury and Health Hazards

BS10EC 381 (U) Mechanism of Action of Anticholinesterases and Anticholinesterase Antidotes

Two classes of acetylcholinesterases (AChE) isoenzymes were isolated from rat cerebrum after a nonlethal, acute exposure (.9 LD_{50}) of Soman. They were inhibited by Soman at different rates even though all forms of AChE were completely inhibited fifteen minutes after exposure. The same isoenzyme profile held true for chronic exposures to Soman.

Preliminary work on identifying sites of action of organophosphorus compounds resulted in the development of a model for evaluating the actions of drugs with highly characterized pharmacological sites of action.

Some progress has been made with regard to the role of AChE and its interaction with its receptor.

Spin label studies indicate that membrane lipids are passive in the change of permeability of the membrane during synaptic transmission.

BS10ED383 (U) Neurotransmitter Systems Interactions: Effects of Anticholinesterases and Treatment Compounds

The effects of acute and chronic exposures to Soman were studied in rats. Acute, sublethal exposures caused a differential degree of increase of ACh and choline (ch) levels in different brain areas, with the cerebral cortex having the highest elevation after 40 minutes. The chronic exposures did not produce any change in ACh and ch levels in any brain areas. However, there was a moderate depression of AChE (25%-40%) in brain stem, midbrain and cerebral cortex at six weeks. If chronic exposures were stepped up (3x), ACh but not ch was elevated in brain stem and cerebellum and then returned to normal. The AChE for these exposures was more severely depressed (45%-75%) in all brain areas.

Summary

We have reported progress on nine work units in three different projects for FY80, during which time we virtually established a new laboratory with an updated mission, new plans and new protocols.

PROJECT 3M162734AH26

MEDICAL DEFENSE AGAINST CHEMICAL AGENTS

TASK AREA AA/WORK UNIT 021

USE OF INVERTEBRATES AS MODEL SYSTEMS FOR INVESTIGATING EFFECTS OF CW AGENTS AND TREATMENT COMPOUNDS ON SINGLE CELLS AND GANGLIA

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY		1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734AH26	AA	021
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)* (U) Use of Invertebrates as Model Systems for Investigating Effects of CW Agents and Treatment Compounds on Single Cells and Ganglia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
012900 Physiology; 002300 Biochemistry; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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	CURRENT 81	1.2	250

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign Intelligence considered

20. PERFORMING ORGANIZATION
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NAME: POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) aplysia (U) AcH (U) AntiChE (U) Organophosphate inhibitors (U) Physostigmine (U) Mecamylamine (U) Benzoquinonium

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess the direct neuronal effects of CW Agents and proposed treatment compounds for the soldier, using invertebrates as a model system.

24. (U) Isolated brain ganglion from gastropod molluscs will be tested. Microelectrodes will be inserted into neurons and drugs applied from a miltibarrel micropipette by either iontophoresis or a pneumatic pump. The responses to drugs will be measured as well as the effects of drugs on the responses to neurotransmitters.

25. (U) 80 04 - 80 09. Effects of physostigmine on three known acetylcholine mediated conductance changes have been examined. The negative logarithm of 50 percent inhibition for blockage of binding of Bungarotoxin to Aplysia ganglia have been determined. Baseine data for effects of soman on the three elementary ACh responses are in progress. Abstract No. 252.8, Effects of Physostigmine and Mecamylamine on the Response to Acetylcholine in Aplysia, 10th Annual Meeting Society for Neuroscience, Vol. 6, Nov 1980.

*Available to contractors upon originator's approval

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PROJECT 3M162734AH26 Medical Defense Against Chemical Agents

TASK AREA AA

WORK UNIT 021 Use of Invertebrates as Model Systems for Investigating Effects of CW Agents and Treatment Compounds on Single Cells and Ganglia

INVESTIGATOR Mrs. Margaret G. Filbert

BACKGROUND

Not all physiological effects exerted by anticholinesterase agents can be explained by mechanisms of cholinesterase inhibition. For the past 35 years, the primary thinking regarding prophylaxis and therapy for nerve agent exposure has been directed at the acute phase of poisoning. The rationale for treatment which has predominated is that both toxicity and incapacitation result from accumulation of acetylcholine at synaptic junctions following inhibition of acetylcholinesterase by nerve agents. This rationale is supported by indirect evidence, such as the efficacy of cholinolytics, oximes and carbamate-prophylaxis in reducing toxic symptomology as well as dose-dependent reduction in lethality; direct evidence validating this position, from in vivo studies, is found to be wanting. Continued reliance upon this rationale, in the absence of an understanding of actual sites and mechanisms of action of both challenge agents and treatment compounds, is unlikely to provide dramatically enhanced treatment regimens for either acute or long-term management of exposure-victims or prophylactic protection for the soldier. Sites and mechanisms of action, as well as their relative contribution to lethality and incapacitation, must be identified. Isolated brain ganglia from gastropod molluscs (Aplysia) were used to examine cholinesterase independent effects of agents and drugs on neurotransmitter responses.

PROGRESS

Effects of physostigmine on ACh responses with different methods of application.

Cells of the right abdominal ganglion, designated as RB cells, were used for these experiments. Cells of the RB group give a depolarizing response to application of ACh. The E_{rev} (equilibrium or reversal potential) for this response is obtained by extrapolation and is approximately -30 mV. Physostigmine (eserine), applied by addition to the solution bathing the preparation, usually leads to amplification of the acetylcholine response magnitude and duration. Since any subsynaptic effects of physostigmine that occur might not always be obvious, and physostigmine effects are difficult to reverse by washing with sea water when applied in the perfusing medium, application of physostigmine by iontophoresis appeared to provide a more certain method of observing a postsynaptic action. Physostigmine contains two secondary amino groups having ionization constants of 7.6×10^{-7} and 5×10^{-13} so that at pH 7.8 (the pH of the sea water used to bathe the preparation) there will be a positive charge on the molecule due to protonation of the amino groups. It seemed feasible, therefore, to apply physostigmine by iontophoresis directly onto the cell membrane or near the receptor site. Figure 1 shows the depolarizing response of an RB cell to iontophoretic application of ACh. A test iontophoretic pulse of physostigmine had no effect on the resting potential of this cell. When a similar pulse of physostigmine was followed by ACh, the response was significantly reduced. After approximately three to five minutes washout with ASW (artificial sea water), a partial recovery of the response amplitude to ACh was seen (figure 1A).

In another experiment the physostigmine was applied by micro-pressure ejection from a micropipette. A pulse of physostigmine at 30 psi followed by a 10^3 na (nanoampere) iontophoretic pulse of ACh completely abolished the response. After several minutes of washout, recovery of the response was again observed (figure 1B).

In a third experiment (figure 1C), the physostigmine was applied in the perfusing ASW. A pre-drug response to iontophoresis of ACh is shown first at an amplifier gain of 4 mV/cm and then at 20 mV/cm. Application of 10^{-6}M physostigmine alone produced an increase in the response. This was partially reversed by 16 minutes of washing with ASW. 10^{-5}M physostigmine was then applied to the chamber and it can be seen that both the amplitude and duration of the ACh response are considerably larger than the pre-drug response. After 60 minutes washout, only partial recovery of the effect of ChE inhibition occurred.

Application of ACh by iontophoretic or micropressure ejection appears to circumvent the effects of inhibition of ChE and clearly shows an effect on the response of the neuronal membrane to ACh that cannot be ascribed to enzyme inhibition.

An experiment similar to the previous one was performed on a cell giving a hyperpolarizing response to ACh. Carbachol was used to mimic the ACh response in this case. It has been shown by Kehoe (1972) that carbachol elicits the identical three elementary responses as does ACh.

A pre-drug response to carbachol is shown in figure 2A. An iontophoretic pulse of physostigmine produced a slight membrane depolarization which returned to the resting level at the end of the pulse. This was shown to be an artifact since reversing the polarity of the current used to eject the drug produced a mirror image of the membrane displacement seen here. When the physostigmine pulse was followed by a carbachol pulse, the response was reduced in amplitude. Recovery to the pre-drug level was obtained after washout of physostigmine.

A similar effect was seen when the physostigmine was applied by pressure ejection. Pressure ejection of the drug had no effect on the resting membrane potential (M.P.). The carbachol response was reduced when preceded by application of physostigmine and recovery was obtained by washout of the drug (figure 2B).

The effects seen in this figure, using carbachol to elicit ACh responses supports the conclusion that the effects of physostigmine are independent of ChE inhibition since (a) carbachol is not hydrolysed by ChE and (b) reversibility is rapid in onset compared to reversibility of the effects with bath applied physostigmine and ACh.

Effects of physostigmine on the three responses to ACh.

The effects of physostigmine on the sodium, chloride and potassium mediated responses to ACh can be seen in figure 3. The RB group 4 cells found on the abdominal ganglian cells are extremely sensitive to ACh and a large depolarizing pre-drug response is seen here. When iontophoretic application of ACh is preceded by an iontophoretic pulse of physostigmine, the depolarizing response to ACh is nearly eliminated. The amplitude recovers with washout in three to five minutes to near pre-drug levels (figure 4A).

In another cell, a rapid hyperpolarization having an E_{rev} for the response at -65 mV was obtained. Application of physostigmine prior to iontophoresis of ACh reduced the response here also. Partial recovery can be seen after washing with ASW (figure 4B).

Still another cell gave a hyperpolarization having a much slower time to peak and prolonged duration. An E_{rev} of -70 mV was determined. This value for the reversal potential along with the slower time course of the response suggested that K^+ was mediating the response to ACh in this cell. Application of physostigmine prior to ACh had little or no significant effect on the response.

It has been shown previously by Kehoe (1972a) that some cells in Aplysia respond to ACh with more than one conductance change. Cell L7 of the left abdominal ganglion produces a two component response that is the result of an increase in sodium conductance followed by a chloride-dependent conductance increase. Iontophoresis of physostigmine prior to carbachol application results in a response with a hyperpolarizing component only (figure 4A). The depolarizing component reappears after washout of the drug.

The left plueral giant cell also gives a two component response to ACh (Kehoe, 1972b). This response is composed of a rapid hyperpolarization followed by a hyperpolarization of much slower time course. The biphasic character of this response is best seen when the membrane potential is held at a level between E_{Cl^-} and E_{K^+}. Figure 4B shows the pre-drug response when the membrane potential is held at -40 mV and also when the membrane potential is at -60 mV. Iontophoretic application of physostigmine immediately before carbachol leaves only the slow component of this response. When the drug is washed out, the rapid chloride component returns.

In summary the effects of physostigmine on three known acetylcholine mediated conductance changes have been examined. We will begin collecting data for the effects of soman on these three responses.

PUBLICATIONS

None.

PRESENTATIONS

TTCP E/TP1, 1979 DRES, Ralston, Canada.

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EFFECTS OF ESERINE ON ACh RESPONSE WITH DIFFERENT METHODS OF APPLICATION

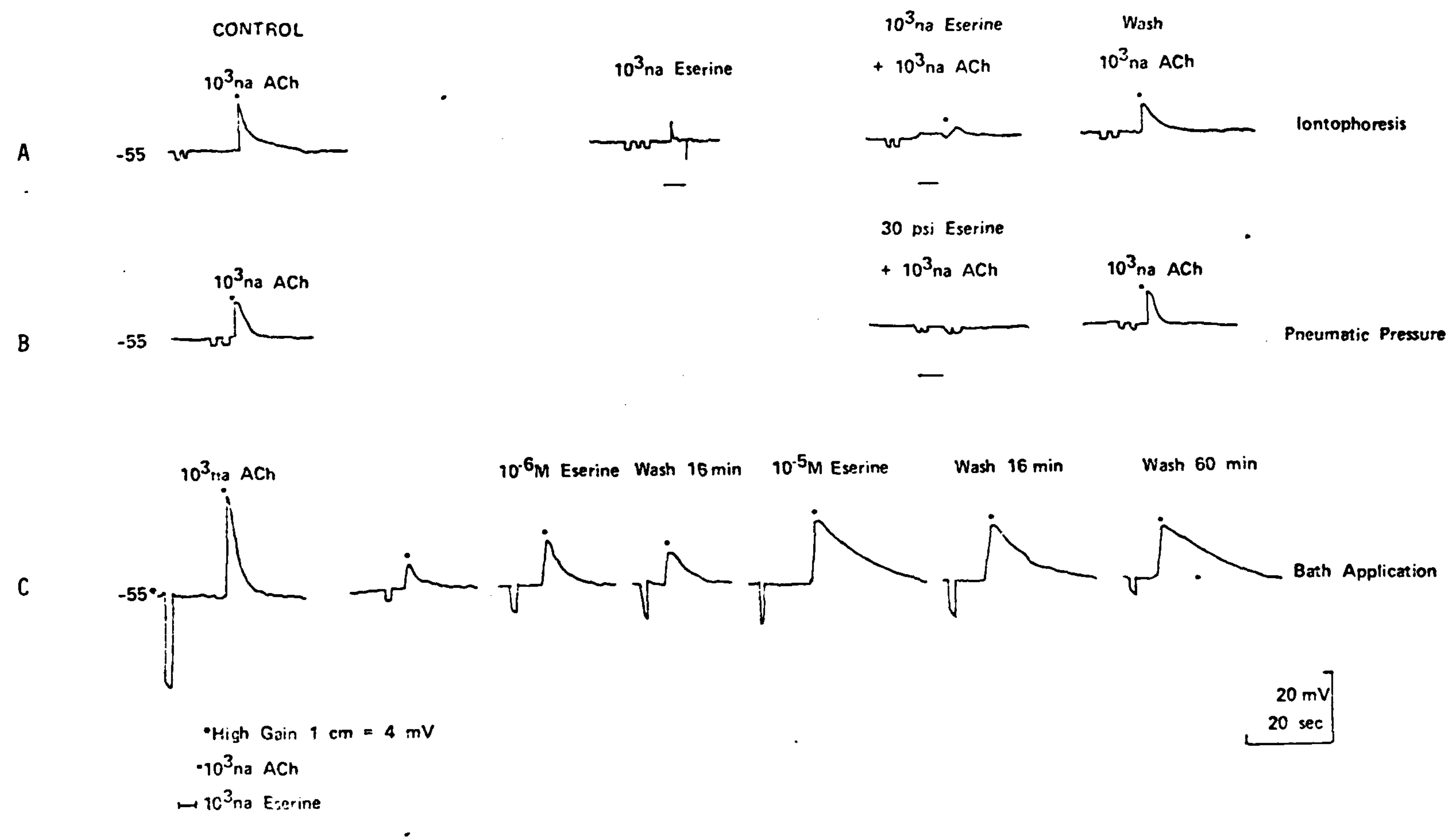

Figure 1

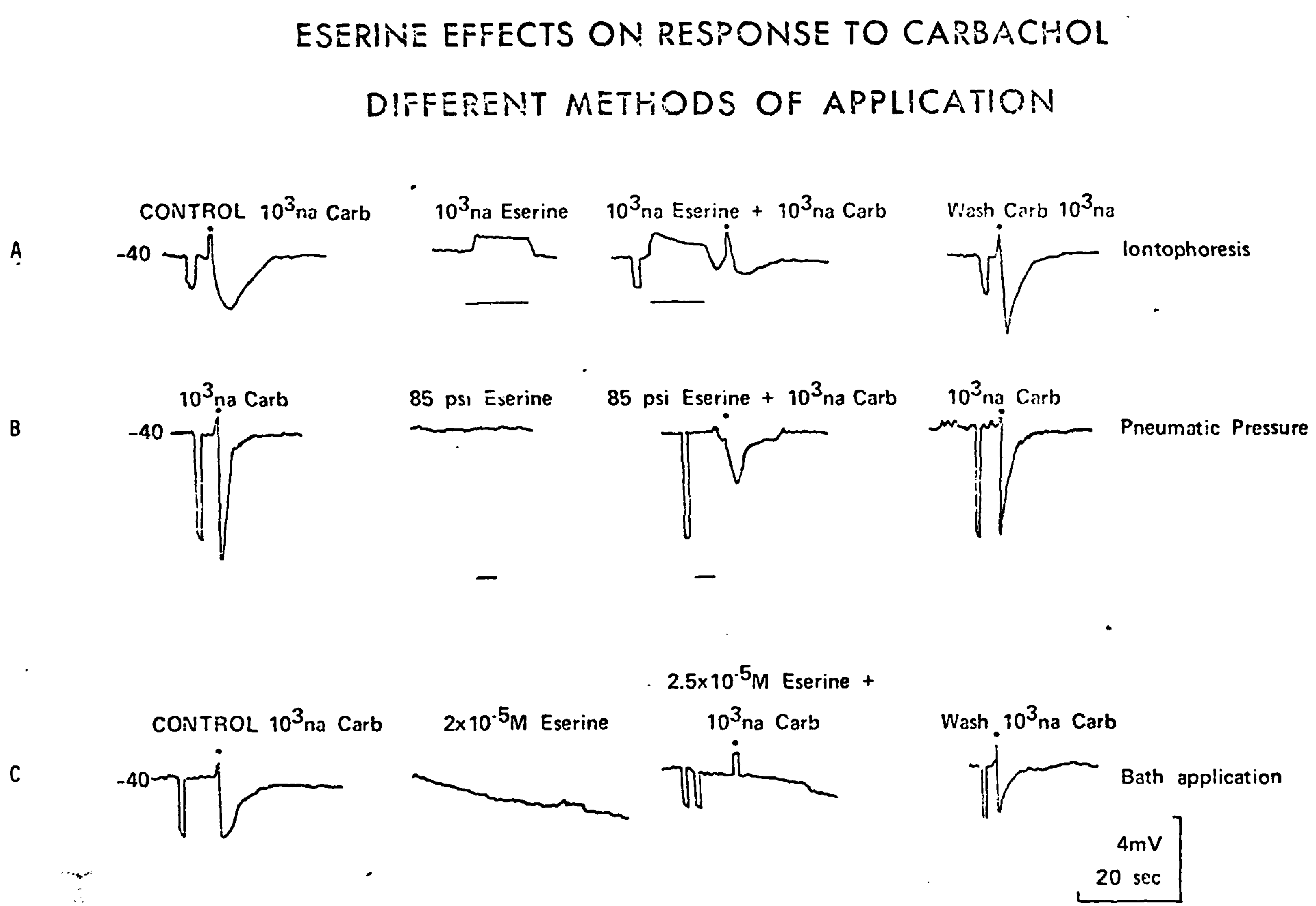
ESERINE EFFECTS ON RESPONSE TO CARBACHOL
DIFFERENT METHODS OF APPLICATION
A
CONTROL 10³na Carb
-40
10³na Eserine
10³na Eserine + 10³na Carb
Wash Carb 10³na
Iontophoresis
B
10³na Carb
-40
85 psi Eserine
85 psi Eserine + 10³na Carb
10³na Carb
Pneumatic Pressure
C
CONTROL 10³na Carb
-40
2x10⁻⁵M Eserine
2.5x10⁻⁵M Eserine +
10³na Carb
Wash 10³na Carb
Bath application
4mV
20 sec

Figure 2

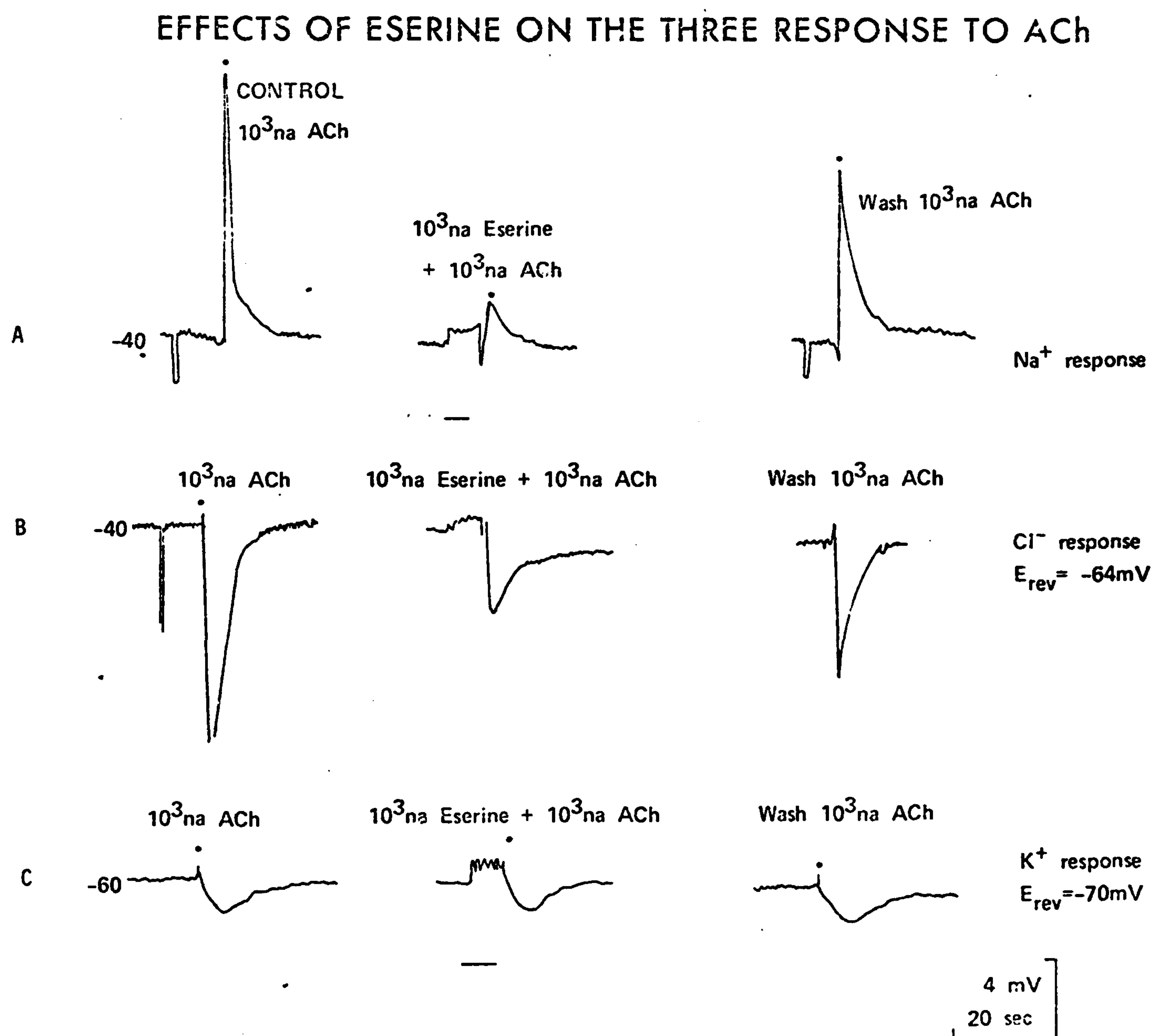
EFFECTS OF ESERINE ON THE THREE RESPONSE TO ACh
A
-40
CONTROL
10³na ACh
10³na Eserine
+ 10³na ACh
Wash 10³na ACh
Na⁺ response
B
-40
10³na ACh
10³na Eserine + 10³na ACh
Wash 10³na ACh
Cl⁻ response
E rev = -64mV
C
-60
10³na ACh
10³na Eserine + 10³na ACh
Wash 10³na ACh
K⁺ response
E rev =-70mV
4 mV
20 sec

Figure 3

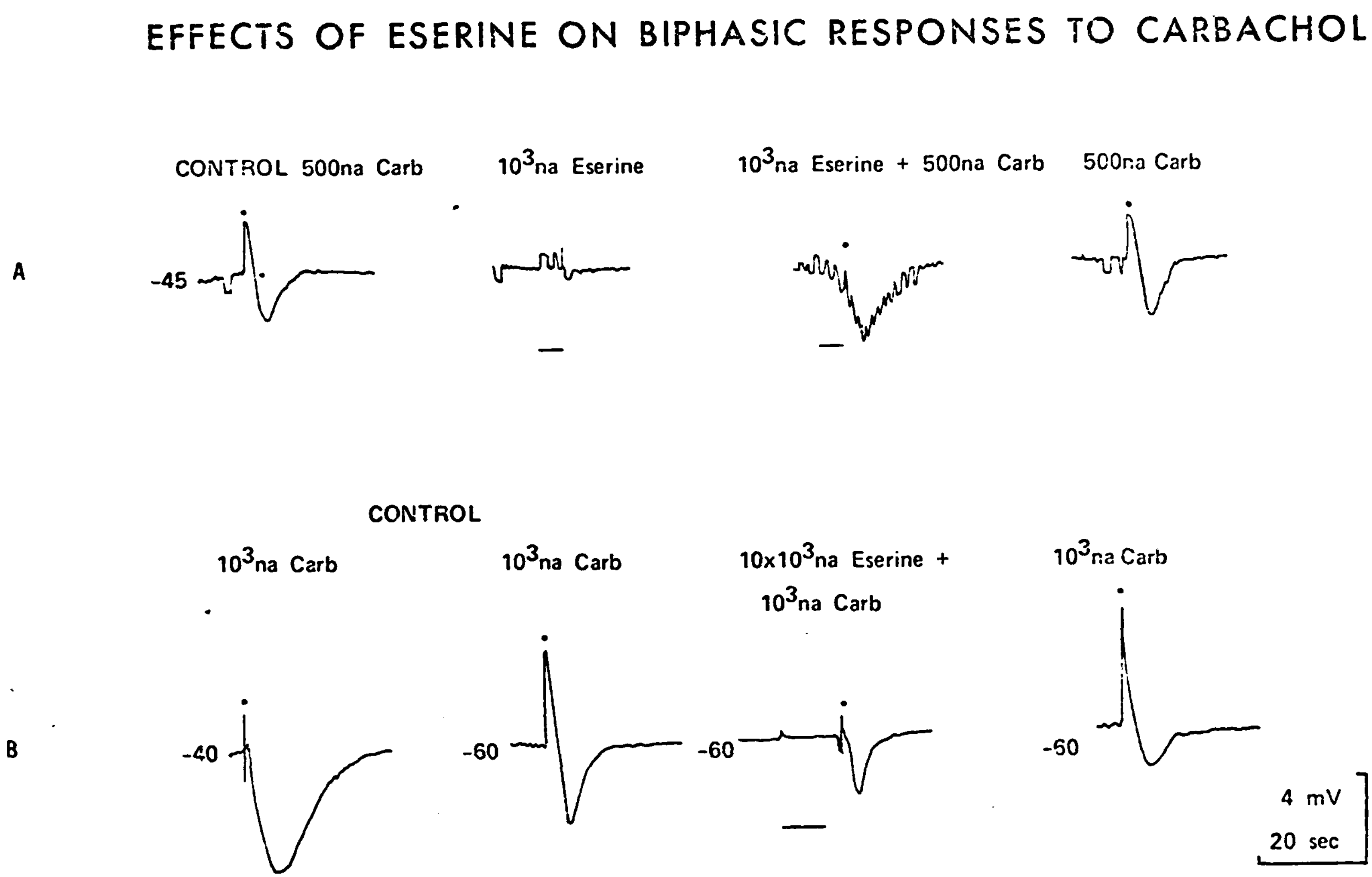
EFFECTS OF ESERINE ON BIPHASIC RESPONSES TO CARBACHOL
A
CONTROL 500na Carb
-45
10^3na Eserine
10^3na Eserine + 500na Carb
500na Carb
B
CONTROL
10^3na Carb
-40
10^3na Carb
-60
$10x10^3$na Eserine +
10^3na Carb
-60
10^3na Carb
-60
4 mV
20 sec

Figure 4

TASK AREA AC/WORK UNIT 024

EFFICACY OF CENTRALLY AND PERIPHERALLY ACTIVE-PRETREATMENT AND TREATMENT COMPOUNDS AGAINST NERVE AGENT INTOXICATION

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG 6517	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734AH26	AC	024
b. CONTRIBUTING				
	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code) (U) Efficacy of Centrally and Peripherally Active-Pretreatment and Treatment Compounds Against Nerve Agent Intoxication

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
012600 Pharmacology; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	4.5	1182
	CURRENT 81	2.5	618

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Biomedical Laboratory
ADDRESS: Aberdeen Proving Ground, MD 21010
RESPONSIBLE INDIVIDUAL
NAME: Llewellyn, C.H.
TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION
NAME: US Army Biomedical Laboratory
ADDRESS: Aberdeen Proving Ground, MD 21010
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Harris, L.W.
TELEPHONE: 301-671-3148
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Lawson, M.A.
NAME: Lennox, W.J. POC:DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Soman, (U) Sarin, (U) Acetylcholinesterase, (U) Acetylcholine, (U) Carbamate, (U) Oximes, (U) Cholinolytics

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop pretreatment/therapy mixtures for the soldier that will not cause mental or physical incapacitation when administered alone but will protect from lethal exposure to nerve agents as well as dampen or abolish agent-induced physical and mental incapacitation and when combined with therapy will prevent death against 3-5 LD50s of nerve agent.

24. (U) Behavioral free nerve agent antidotal mixtures for rats and marmosets will be developed. Pretreatment/therapeutic antidotes are used to antagonize agent-induced lethality and physical and mental debilitation. Biochemical studies are also run to see if there is a relationship between agent-induced incapacitation and brain AChE activity and/or ACh levels. Guinea pigs will be used in pretreatment/therapy studies to see whether N-hydroxy carbamates are as effective as physostigmine against Soman; pharmacology of mixtures also will be assessed.

25. (U) (80 04-80 10) Two "behavioral free" pretreatment mixtures (physostigmine (Ph) or pyridostigmine (Py) plus atropine and mecamylamine) have been developed for rats. Both mixtures are equally effective against agent lethality. The Ph containing mixture was found to be markedly superior to the Py mixture in abolishing Soman-induced physical and mental debilitation. This work was presented at the Pharmacology (ASPET) meeting, August 1980. A manuscript was also published on efficacy of chemical pretreatment against Soman (Life Sciences 26, 1885 (1980).

DD FORM 1498

PROJECT 3M162734AH26 Medical Defense Against Chemical Agents

TASK AREA AC

WORK UNIT 024 Efficacy of Centrally and Peripherally Active-Pretreatment and Treatment Compounds Against Nerve Agent Intoxication

INVESTIGATORS Larrel W. Harris
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David L. Stitcher
Willard J. Lennox

BACKGROUND

Conventional therapy will protect against lethality from most agents but exposed subjects are likely to be incapacitated for up to several days. Consequently,this would be unacceptable on a chemical battlefield because the soldier must be able to continue the military mission. Ensuring a functional soldier can best be accomplished with physical protection (mask and clothing) and appropriate chemical pretreatment. Therefore, the Army needs to develop a pretreatment antidote which when given alone will not adversely affect performance, but will provide reasonable protection from both agent-induced lethality and physical and mental debilitation, and in case of serious exposure will delay death for sufficient time to allow appropriate treatment and/or evacuation.

In the past, emphasis was placed on reducing agent lethality. The dose of therapeutic or prophylactic drugs chosen was that which provided the greatest survival from nerve agent intoxication.

Recently, we have been reassessing the approach to take in dealing with chemical pretreatment/therapy against nerve agent poisoning. It is expected that troops will be schooled on the possibility of having nerve agents used against them on a future battlefield; they will also know what signs and symptoms to expect upon exposure. However, it is possible that tensions and anxieties on the battlefield might lead some troops to imagine that they had been exposed to nerve agent. An alarm by frightened soldiers could lead large numbers of troops to give themselves the nerve agent self-aid antidote,which in itself might cause temporary incapacitation. On the other hand, if troops wait until they have unmistakable symptoms of nerve agent exposure, those which are in concentrated agent pockets will, probably, rapidly experience severe and debilitating symptoms which may be so intense that they will be unable to both mask and administer the self-aid antidote. Moreover if they are able to administer conventional atropine/oxime therapy, it is likely that they will be severely incapacitated for several hours and even days. For these reasons we have been directing our attention to sign-free injectable pretreatment mixtures with the thought in mind that these mixtures should meet guidelines outlined in figure 1.

We are, therefore, presently leaning toward the idea that drug formulations for rats and monkeys must be based on criteria that are proposed for use in man, i.e., behavioral titration vs. survival titration (which cannot be used in man). This means that responses other than survival must be used in selection of doses in experimental animals as well as in man, such as physical incapacitation, performance decrement and changes in behavioral stresses.

In the past, emphasis has been placed on the carbamate pyridostigmine. However, pyridostigmine has been shown to have a serious deficiency in that while it will protect against lethality,it appears to be inefficient in antagonizing agent-induced physical and mental debilitation. Central nervous system acting pretreatment/therapy drugs (carbamates and/or oximes) are theoretically of much greater field potential, because they would be expected to manage central acetylcholine levels and thus prevent the prolonged psychiatric sequellae that can follow exposure to nerve agents.

PROGRESS

Adjuncts in carbamate prophylaxis against soman.

Carbamates are very effective in protecting animals from the lethal effects of soman (table 1). Atropine + pyridostigmine pretreatment increased the protective ration (LD_{50}, pretreated/LD_{50}, untreated) from 1 (soman only) to 6.2. The inclusion of mecamylamine in the drug regimen further elevated the protective ratio to 23.8.

Decrement "Free" Pretreatment Mixtures (Mix I and Mix II).

We have been successful in developing two such mixtures. They cause little or no physical incapacitation as measured by an accelerating rotarod, or mental incapacitation as measured by a two-component operant schedule-FR10 schedule for milk rewards for 20 minutes followed by a non-cued 10 minute period of extinction (no rewards). The composition of the two mixtures are given in table 2.

Efficacy of pretreatment mixtures against soman and DFP.

Since CW nerve agents can only be used at CW establishments, and since diisopropylfluorophosphate (DFP) has been considered by other investigators as a model for these agents, it was crucial to identify any differences in response of animals exposed to DFP and soman (alone and together with chemical pretreatment). These two agents are considered irreversible inhibitors of the cholinesterase enzymes in that no spontaneous dephosphorylation occurs following exposure.

The protective ratios for Mix I and Mix II pretreated rats exposed to soman and DFP are summarized in table 3. Both mixtures are effective against the lethal effects of these agents. Furthermore, the protection offered by these mixtures is considerably higher in DFP exposed rats. Visual inspection of the 24-hour survivors in the above studies revealed that the quality of life in Mix II protected rats were far superior to those rats protected with Mix I. Because of these observations we set out to study the effects of chemical pretreatment on agent-induced incapacitation. We were aware that others had previously used the accelerating rotarod test to study both agent- and drug-induced physical incapacitation. As a result we utilized this test to assess agent-induced physical incapacitation in DFP and soman exposed rats. In brief, pretreatment drugs were given IM to male rats (180-210 g) 30 minutes before challenging with soman or DFP intravenously (IV). These animals were tested on the rotarod 1/2, 1, 2 and 24-hours post agent. The following animals were always tested together on the rotarod: saline control, Mix I or Mix II, Mix I or Mix II + agent, and agent only. The agent alone group were run as a check on the potency of the sample being tested enabling comparisons to be made with earlier data. All animals received the same number of injections by the same routes, receiving saline for each drug administered. The degree of incapacitation was determined by means of the incapacitation ratio (IR) which is:

$$\frac{ud}{uc + ud}$$

where ud = mean duration of stay on the accelerating rotarod for drug animals and

uc = mean duration of stay on the rotarod for control animals.

Time scores from control and experimental groups were compared for significance using a student's T'test.

The effects of various doses of soman on physical incapacitation (as IR approaches 0, the more pronounced is the incapacitation), are shown in figure 2. 0.79 LD_{50} of soman causes physical incapacitation and that incapacitation becomes progressively worse as the level of agent is increased. By plotting an incapacitation ratio (IR) (abscissa vs. the LD_{50} of soman injected on the ordinate) we were able to estimate the dose of soman required to produce 50% incapacitation at 30 minutes. This was 0.84 LD_{50}. The maximum sign-free dose of soman was estimated in a similar manner to be 0.55 LD_{50}.

Figure 3 illustrates the protective effects of Mix I. This pretreatment appears to be effective up to and including 1 LD_{50}. Even though pretreatment completely protected rats from the lethal effects of 1.26 LD_{50} of soman, incapacitation was severe and persisted for up to 2 or more hours. At a 1.59 LD_{50} challenge, animals were almost totally incapacitated even after 24 hours.

The contrast between Mix I and Mix II in antagonizing soman-induced physical incapacitation is expressed in figure 4. Physostigmine protects both peripheral and brain AChE from inhibition by soman. Pretreatment with Mix II completely reversed agent-induced physical incapacitation by 30 minutes at challenges of soman up to and including 1.59 LD_{50}.

<u>AChE levels in protected rats exposed to soman (IV).</u>

Brain AChE levels in protected rats exposed to 1.3 LD_{50} of soman are shown in table 4. While AChE activity in the brain is much higher in Mix II-protected animals, peripheral AChE activity is similar in both groups of animals.

<u>Effects of DFP on incapacitation.</u>

The effects of various doses of DFP on physical incapacitation (PI) are shown in figure 5. DFP causes a PI at lower doses than soman. For instance, as little as 0.4 LD_{50} of DFP causes significant incapacitation. As with soman, DFP-induced PI persists at doses higher than 0.63 LD_{50}.

Figure 6 illustrates the protective effects of Mix I against DFP-induced PI. The data show that chemical pretreatment of rats with Mix I is only marginally effective against DFP-induced PI. At challenges higher than 0.4 LD_{50}, PI persisted for the first two hours, but by 24 hours, animals had recovered, except for those challenged with 2.5 LD_{50} DFP.

Figure 7 shows the protective effects of Mix II in antagonizing DFP-induced PI. At challenges higher than 1.58 LD_{50}, PI persisted for at least 2 hours; when compared to PI at 30 minutes, the degree of incapacitation appears less severe at two hours. However, by 24 hours all animals had recovered.

Brain and peripheral acetylcholinesterase (AChE) levels in protected rats.

AChE levels of protected rats exposed to 1.3 LD_{50} of DFP can be seen in table 5. As with soman, brain AChE activity is much higher in Mix II protected rats.

Summary.

(1) Both DFP and soman induce PI.

(2) Chemical pretreatment with a single level of Mix I is only slightly effective against agent-induced physical incapacitation.

(3) Chemical pretreatment with the physostigmine containing mixture (Mix II) is highly effective against both soman and DFP-induced physical incapacitation and lethality. For instance the protective ratio against DFP is 6.9.

(4) The fact that brain AChE activity is higher in Mix II (physostigmine) than in Mix I protected animals reflects the central actions of physostigmine. The debilitation observed in Mix I (phyriostigmine) protected animals is probably due to excess acetylcholine resulting from marked inhibition of brain AChE.

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TABLE 1

ADJUNCTS IN CARBAMATE PROPHYLAXIS AGAINST SOMAN IN RABBITS

TREATMENT	PROTECTIVE RATIO
PYRIDOSTIGMINE + ATROPINE	6.2
PYRIDOSTIGMINE + ATROPINE + MECAMYLAMINE	23.8

TABLE 2

CHEMICAL PRETREATMENT MIXTURES

MIX 1* =	ATROPINE SULFATE	0.79 MG/KG
	MECAMYLAMINE	0.79 MG/KG
	PYRIDOSTIGMINE	0.056 MG/KG
MIX II** =	ATROPINE SULFATE	0.79 MG/KG
	MECAMYLAMINE	0.79 MG/KG
	PHYSOSTIGMINE	0.026 MG/KG

*MIX 1 AND MIX II CAUSED NO BEHAVIORAL CHANGES IN TRAINED RATS IN BEHAVIORAL STUDIES (FIXED RATIO/EXTINCTION). THE MIXTURES WERE INJECTED IM 30 MIN PRIOR TO SOMAN (GD).

TABLE 3

PROTECTIVE EFFECTS OF MIX I AND MIX II AGAINST SOMAN AND DFP EXPOSURE IN RATS

PRETREATMENT*	LD_{50} MEAN (95% CONFIDENCE LIMITS)	PROTECTIVE** RATIO
SOMAN, µg/kg	61.0 (53.6 – 69.3)	
MIX I + SOMAN	107.9 (82.0 – 142.3)	1.8
MIX II + SOMAN	140.0 (123.7 – 158.4)	2.3
DFP, mg/kg	1.3 (1.1 – 1.4)	
MIX I + DFP	3.6 (3.0 – 5.9)	2.8
MIX II + DFP	9.0 (7.9 – 10.3)	6.9

* ANIMALS WERE ADMINISTERED PRETREATMENT MIXTURES IM 30 MINUTES BEFORE EXPOSURE TO AGENT, IV

** PROTECTIVE RATIO = $\frac{LD_{50}\text{ (WITH PRETREATMENT)}}{LD_{50}\text{ (UNTREATED)}}$

TABLE 4

ACETYLCHOLINESTERASE ACTIVITY IN MIX I AND MIX II PROTECTED RATS EXPOSED TO SOMAN*

TISSUE	ACETYLCHOLINESTERASE ACTIVITY % CONTROL ± S.D.	
	MIX I	MIX II
CEREBRAL HEMISPHERE	15.3 ± 8.3	46.3 ± 5.1
BRAIN STEM	17.5 ± 2.7	56.4 ± 7.2
DIAPHRAGM	46.7 ± 17.0	49.3 ± 9.0
BLOOD	66.4 ± 4.0	65.3 ± 5.9

* 1.3 LD_{50} SOMAN, IV

TABLE 5

ACETYLCHOLINESTERASE ACTIVITY IN MIX I AND MIX II PROTECTED RATS EXPOSED TO DFP*

TISSUE	ACETYLCHOLINESTERASE ACTIVITY % CONTROL ± S.E.	
	MIX I	MIX II
CEREBRAL HEMISPHERE	7.0 ± 0.6	28.3 ± 1.5
BRAIN STEM	17.2 ± 1.2	34.7 ± 2.3
DIAPHRAGM	51.2 ± 1.9	41.9 ± 3.1
BLOOD	49.3 ± 3.2	38.5 ± 2.9

*1.3 LD_{50} DFP, IV

PROPHYLAXIS AND THERAPY AGAINST NERVE AGENTS

CHEMICAL PRETREATMENT*	THERAPY**
PILLS or AUTOINJECTOR	PILLS or AUTOINJECTOR
YES	AS NEEDED

PRETREATMENT MIXTURE –

1. SHOULD NOT IMPAIR PERFORMANCE OF SUBJECTS WHEN ADMINISTERED ALONE.
2. SHOULD SAVE ALL SUBJECTS FROM AN "LD_{100}" DOSE OF AGENT.
3. SHOULD PREVENT DEBILITATION FROM AN LD_{50} DOSE OF AGENT.
4. SHOULD DAMPEN SIGNS OF SEVERE AGENT EXPOSURE AND SUPPORT LIFE FOR SUFFICIENT TIME TO ALLOW ADMINISTRATION OF THERAPY.
5. WHEN SUPPLEMENTED WITH THERAPY, SHOULD PROTECT SUBJECTS FROM AT LEAST 5 LD_{50}s OF AGENT.

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*SELF ADMINISTRATION

**ADMINISTERED BY MEDICAL PERSONNEL

Figure 1

SOMAN (GD) - INDUCED PHYSICAL INCAPACITATION IN RATS

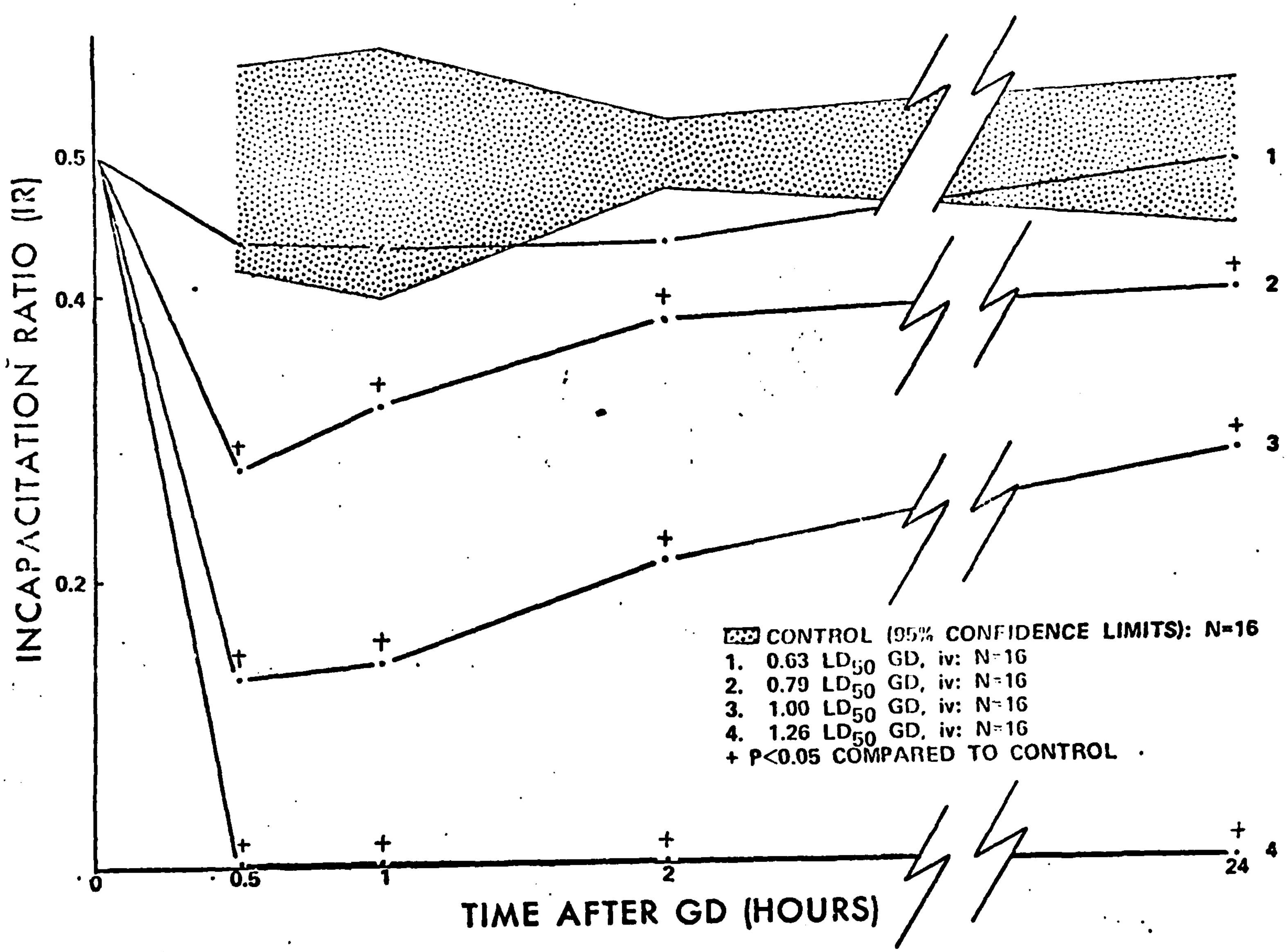

Figure 2

CHEMICAL PRETREATMENT: EFFECTS ON SOMAN (GD) - INDUCED PHYSICAL INCAPACITATION

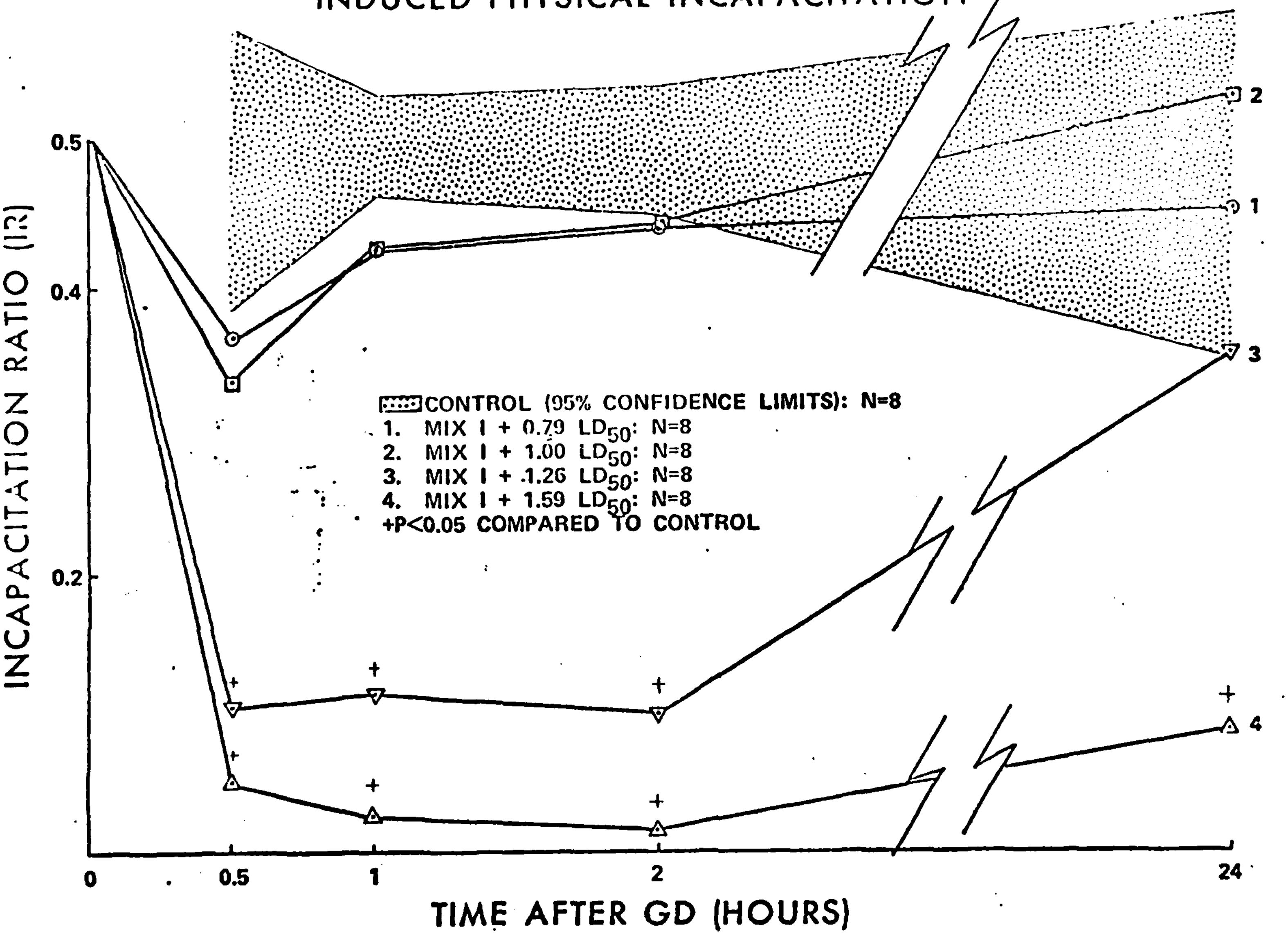

Figure 3

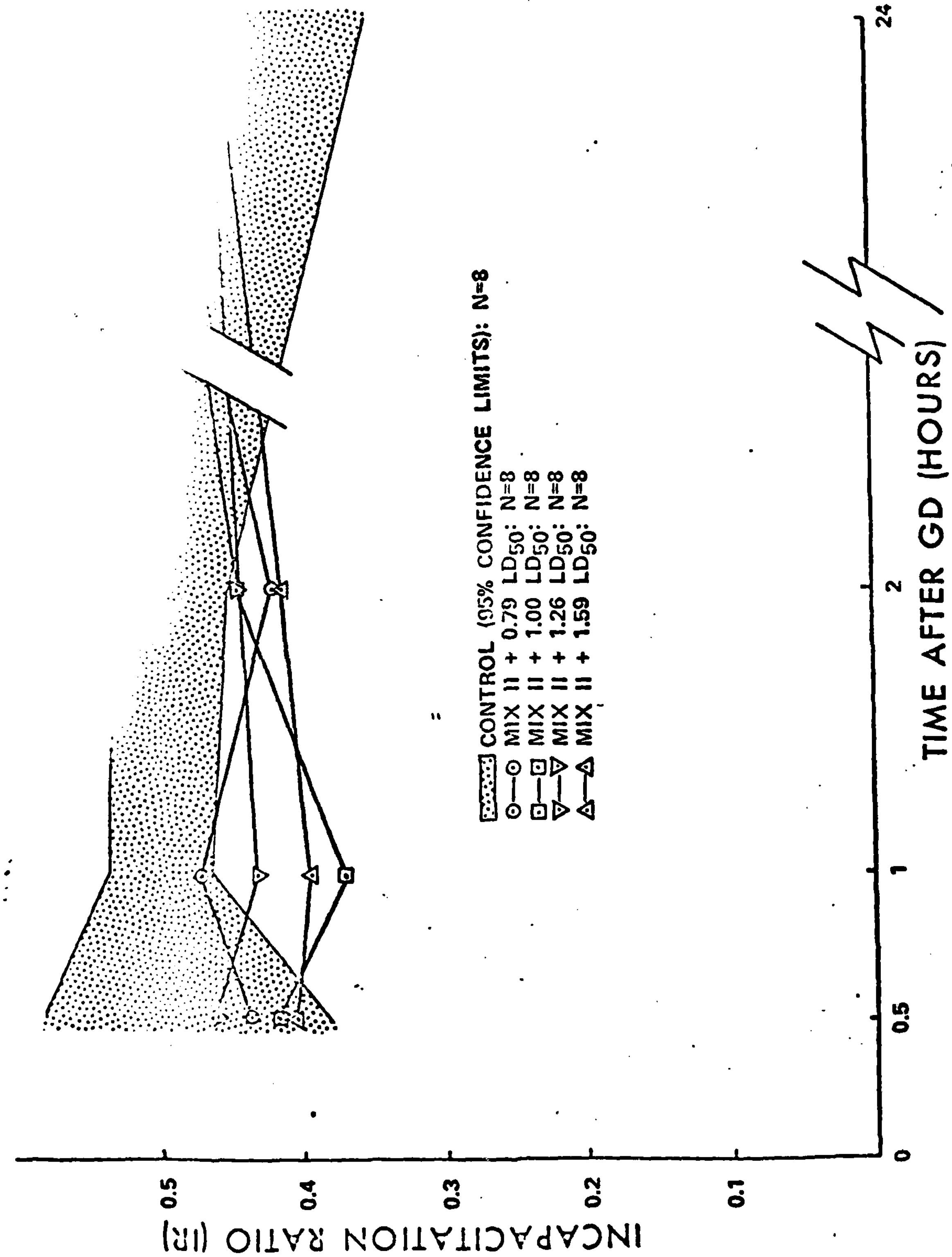
INCAPACITATION RATIO (IR)
0.5
0.4
0.3
0.2
0.1
0
0.5
1
2
24
TIME AFTER GD (HOURS)
CONTROL (95% CONFIDENCE LIMITS): N=8
MIX II + 0.79 LD50: N=8
MIX II + 1.00 LD50: N=8
MIX II + 1.26 LD50: N=8
MIX II + 1.59 LD50: N=8

Figure 4

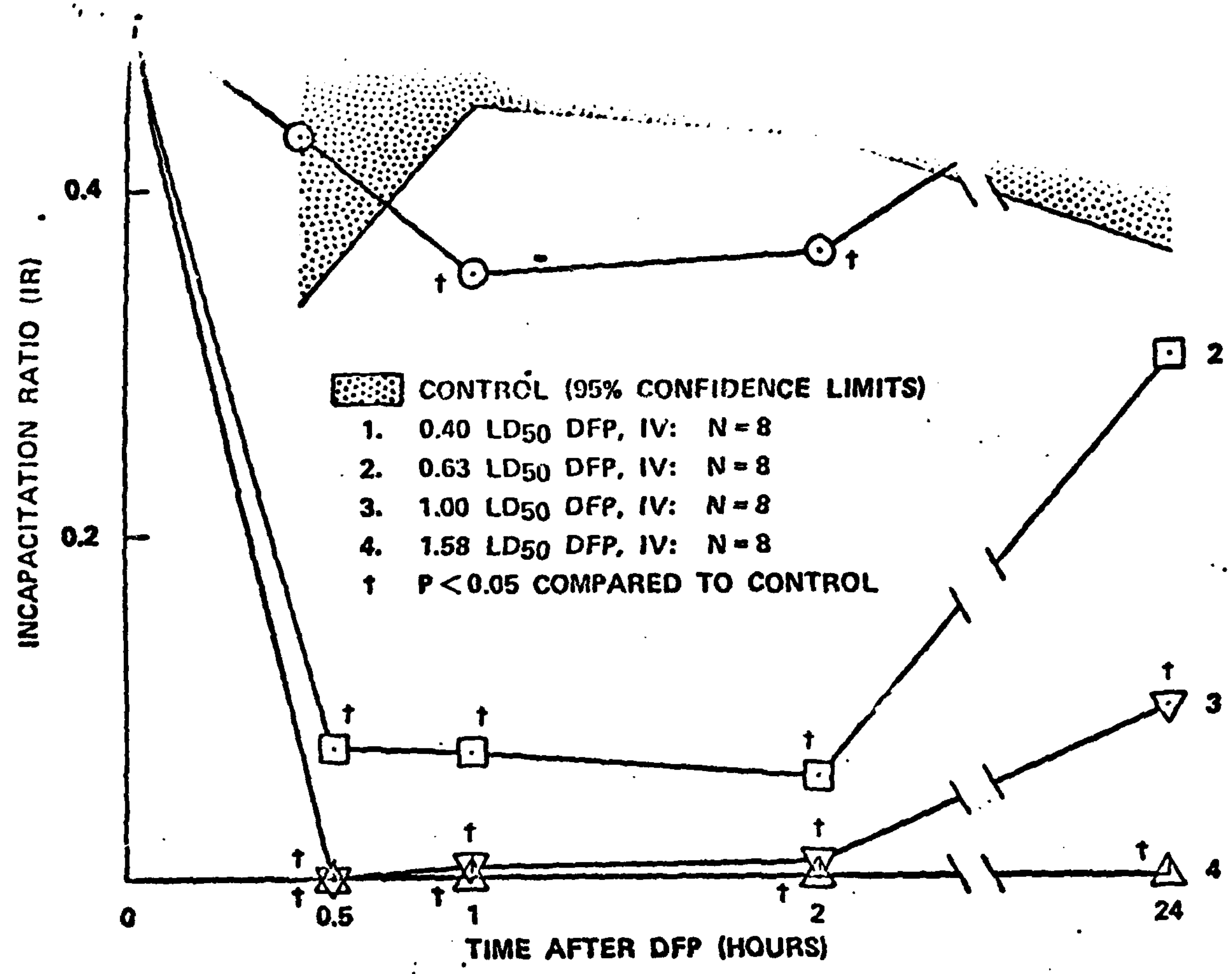
INCAPACITATION RATIO (IR)
0.4
0.2
0
0.5
1
2
24
TIME AFTER DFP (HOURS)
CONTROL (95% CONFIDENCE LIMITS)
1. 0.40 LD50 DFP, IV: N = 8
2. 0.63 LD50 DFP, IV: N = 8
3. 1.00 LD50 DFP, IV: N = 8
4. 1.58 LD50 DFP, IV: N = 8
† P < 0.05 COMPARED TO CONTROL
2
3
4

Figure 5

CHEMICAL PRETREATMENT:

EFFECTS ON DFP-INDUCED PHYSICAL INCAPACITATION

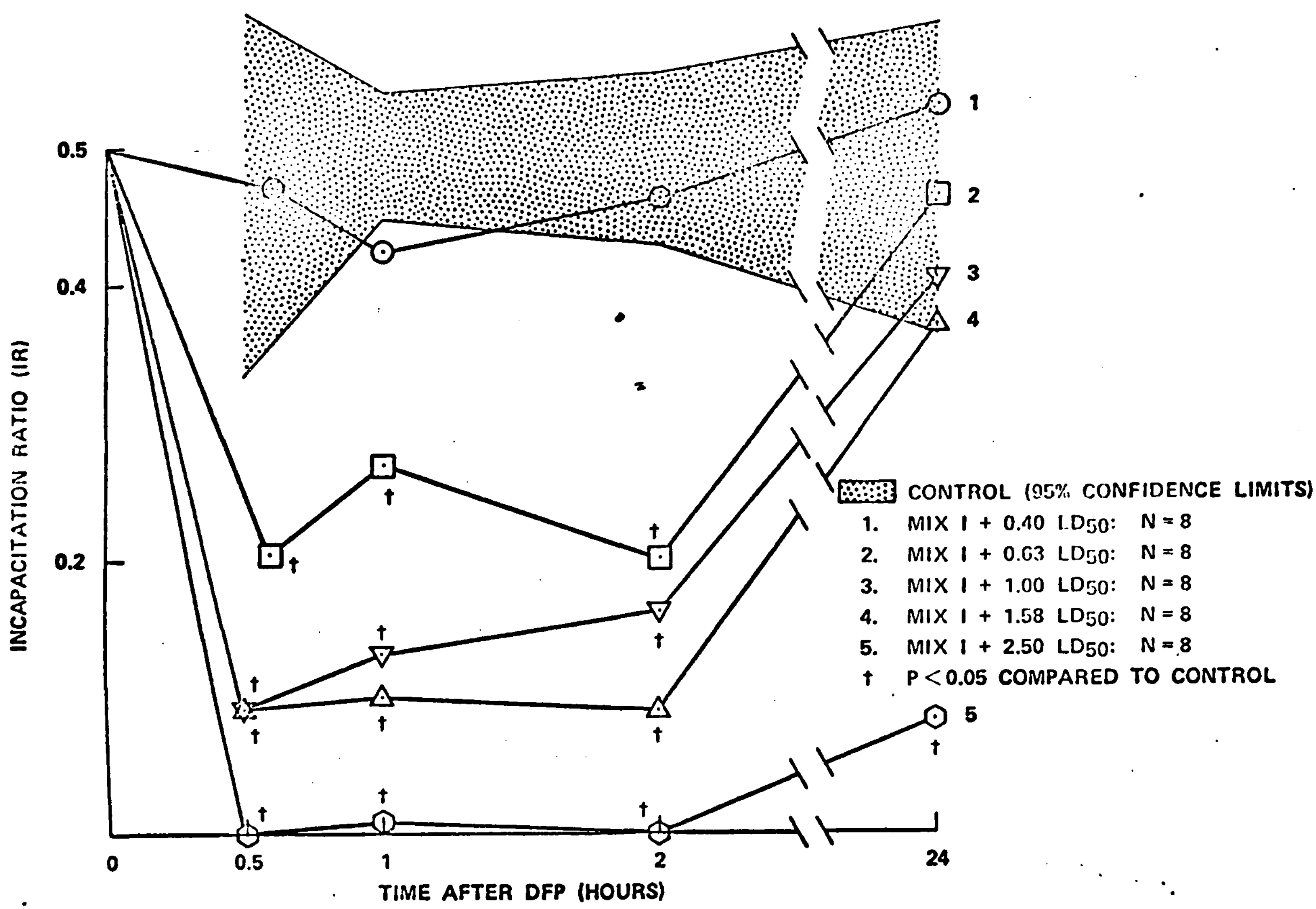

Figure 6

CHEMICAL PRETREATMENT:
EFFECTS ON DFP-INDUCED PHYSICAL INCAPACITATION

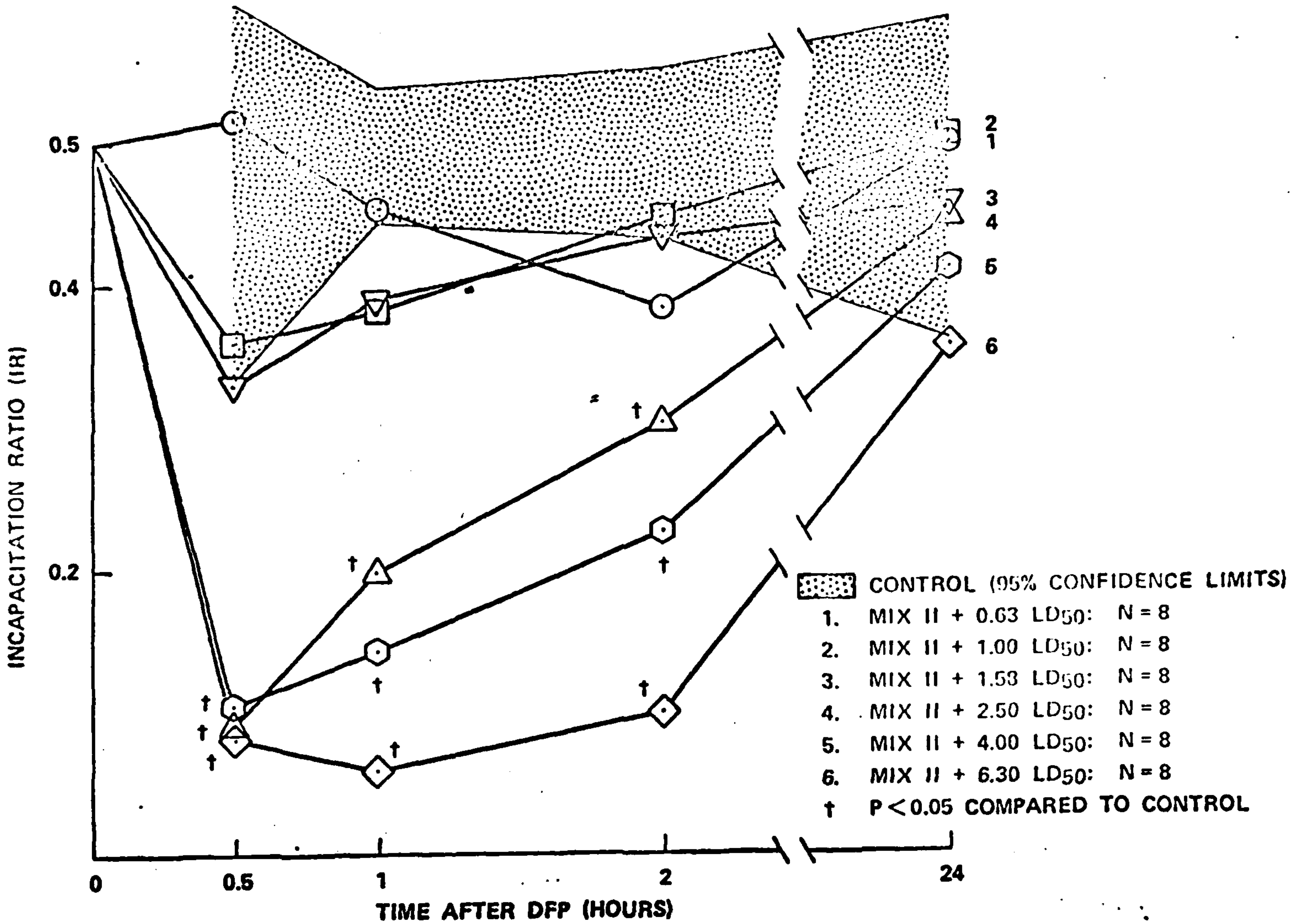

Figure 7

TASK AREA AC/WORK UNIT 025

COMPARISON OF 4DMAP, SODIUM NITRITE, AMYL NITRITE
AND SODIUM THIOSULFATE: EFFICACY OF TREATMENT
IN ACUTE CYANIDE POISONING

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG 6509	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. Change	U	U		NL	YES / NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A826	AC	025
b. CONTRIBUTING				
xxxxxxxxx		STOG 80-7.2:1		

11. TITLE (Precede with Security Classification Code) (U) Comparison of 4DMAP, Sodium Nitrite, Amyl Nitrite and Sodium Thiosulfate: Efficacy of Treatment in Acute Cyanide Poisoning.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	5.0	1120
	CURRENT 81	3.5	825

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Biomedical Laboratory

ADDRESS: Aberdeen Proving Ground, MD 21010

RESPONSIBLE INDIVIDUAL

NAME: Llewellyn, C.H.

TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION

NAME: US Army Biomedical Laboratory

ADDRESS: Aberdeen Proving Ground, MD 21010

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Hawkins, S.F.

TELEPHONE: 301-671-2216

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Stemler, F.W.

NAME: Johnson, R.P.

POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) cyanide (U) methemoglobin (U) amyl nitrite (U) sodium nitrite (U) 4-DMAP

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess the relative efficacy of the various current treatment regimens for cyanide poisoning.

a. Amyl nitrite plus sodium nitrite plus sodium thiosulfate
b. 4-dimethylaminophenol + sodium thiosulfate
c. synthetic methemoglobin + sodium thiosulfate

In addition, explore other possible mechanisms of action of cyanide and pertinent treatment compounds.

24. (U) Animals (cynomolgus monkeys or dogs) will be exposed to lethal concentrations of cyanide. The animals will then be treated with various combinations of therapeutic compounds. Various physiological parameters will be measured to determine efficacy and extent of side effects, if any.

25. (U) 8004 - 8009. The effective dose of sodium nitrite and 4-DMAP which produces the recommended 30-40% methemoglobin necessary for animal survival has been determined. The lethal dose response curve to cyanide is being established. These data will be the basis for doses of cyanide and treatment compounds that will be used in the efficacy studies.

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PROJECT 3M162734AH26 Medical Defense Against Chemical Agents

TASK AREA AC

WORK UNIT 025 Comparison of 4-DMAP, Sodium Mitrite, Aayl Nitrite, and Sodium Thiosulfate; Efficacy of Treatment in Acute Cyanide Poisoning

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BACKGROUND

It has always been thought that the formation of methemoglobin was the key factor in providing protection against cyanide poisoning. Therefore, most of the antidotes known today are compounds that have the ability to oxidize *in vivo* hemoglobin to methemoglobin. Methemoglobin is thought to compete with cytochrome oxidase for cyanide ions. Amyl nitrite, sodium nitrite and 4-DMAP all oxidize hemoglobin to methemoglobin. It is commonly thought that the rate of methemoglobin formation is a linear function of efficacy. However, this is not necessarily true. There may be other mechanisms of protection.

PROGRESS

Parameters chosen for comparing 4-DMAP and sodium nitrite.

There were five key comparisons used to compare 4-DMAP and sodium nitrite as parenteral therapy in cyanide poisoning.

Rate of methemoglobin production

Respiratory rate

Maintenance of blood pressure

Effect on heart rate and survival rate

These parameters were chosen to compare the relative effectiveness of 4-DMAP and sodium nitrite for the following reasons:

The rate of methemoglobin production is considered to be paramount in the treatment of cyanide poisoning. Cyanide acts very rapidly and causes death within a matter of minutes. Therefore, the treatment has to provide its protection within the same time frame.

Data from literature indicates that cyanide acts primarily at the respiratory centers in the CNS causing cessation of respiration within seconds. It should, therefore, be expected that therapy compounds either enhance respiration or at the very least have no adverse effects on respiration.

Heart rate and blood pressure are obvious parameters to measure, especially since sodium nitrite has been reported to cause orthostatic hypotension.

From the above it is hoped that efficacy will be determined by a comparison of each compound as a therapeutic agent. For the purpose of this study efficacy is defined as the survival rate with respect to time. Therefore, the longer an animal survives with a particular treatment, the more efficacious the treatment is considered to be.

Routes of administration.

It was intended at the beginning of this project that 4-DMAP would be used as an antidote for cyanide poisoning in the field as a self-help or buddy aid. Therefore, in all experiments 4-DMAP was injected intramuscularly. However, since sodium nitrite is administered intravenously in therapy, it was administered in this fashion in all experiments. Using the two compounds in this fashion would render a real life comparison. It should be noted that 4-DMAP, when given intravenously, has been proven effective as treatment for cyanide intoxication.

Rates of methemoglobin formation.

Table 1 shows the rates of methemoglobin formation by various concentrations of 4-DMAP and sodium nitrite (20 mg/kg). The data show that 4 mg/kg of 4-DMAP produces approximately 28% methemoglobin in 10 minutes, while it takes 20 mg/kg of sodium nitrite to produce the same amount in approximately 30 minutes. Maximal methemoglobin levels were observed at thirty and ninety minutes post-injection, with 4-DMAP and sodium nitrite, respectively.

Although control animal experiments were performed with 4 and 6 mg/kg IM 4-DMAP, the dose for obtaining 30-40% methemoglobin was selected as 5 mg/kg for therapeutic experiments.

Maximal effects of 4-DMAP and sodium nitrite.

The data in table 2 show the effects of 4-DMAP (4 mg/kg) and sodium nitrite (20 mg/kg) on respiratory rate/min, heart rate (beats/min) and blood pressure (mm Hg). Neither 4-DMAP nor sodium nitrite affects the respiratory rate. Both sodium nitrite and 4-DMAP result in an increase in heart rate. Sodium nitrite causes a slightly higher increase than 4-DMAP. Sodium nitrite causes a large depression in arterial blood pressure while 4-DMAP causes a less severe depression. It should be noted that when animals are poisoned with cyanide, these depressions in blood pressure are not observed as a result of either treatment.

The current US and proposed therapy consist of a methemoglobin former (sodium nitrite, amyl nitrite and 4-DMAP) and a cyanide scavenger (sodium thiosulfate) to effect the elimination of cyanide from the body. However, this data only addresses the comparison of the methemoglobin formers, 4-DMAP and sodium nitrite.

Relative efficacy of 4-DMAP and sodium nitrite.

The relative efficacy of 4-DMAP and NaNO2 with respect to time was compared in only a limited number of cyanide exposed animals. The control animal, exposed to 2 LD_{50} of sodium cyanide died in 9 minutes. Animals exposed to 2 and 3 LD_{50} NaCN survived after treatment with 4-DMAP or sodium nitrite. It should be noted that 4-DMAP prolongs the life of the animal for a longer period of time than does sodium nitrite with exposure to 3.5 LD_{50} of NaCN. During this period, certainly subsequent therapy could be rendered to save an animal. At 4 LD_{50} of sodium cyanide,neither treatment is effective for any length of time. So the difference in efficacy between 4-DMAP and sodium nitrite appears to be in the very narrow limits between 3 and 3.5 LD_{50}.

Summary.

The preliminary data collected thus far suggests that neither 4-DMAP nor sodium nitrite affects the respiratory rate. Both 4-DMAP and sodium nitrite cause an increase in heart rate, but the increase with sodium nitrite is much greater. Sodium nitrite results in a large depression in blood pressure while 4-DMAP causes a rather mild depression of blood pressure.

Since both treatments appear to save the animals at 3 LD50 exposures, a closer look is necessary to discern the subtle differences between 4-DMAP and sodium nitrite. Also, the comparison of 4-DMAP and sodium nitrite in combination with thiosulfate is necessary. Sodium thiosulfate increases the elimination of cyanide from the body. When used in conjunction with 4-DMAP or sodium nitrite,it may enhance the efficacy of either 4-DMAP or sodium nitrite or both,to higher levels than experienced with either alone.

It is my opinion that before IND studies are started, toxicology and formulation stability studies should be completed. There are presently insufficient data on acute toxicity, chronic toxicity and carcinogenic properties of 4-DMAP. At present 4-DMAP is only manufactured in the IV formulation. Stability studies are needed for the im formulation. It is known that 4-DMAP reacts with metals and certain kinds of rubber. Studies should also be performed with injectable container materials.

PUBLICATIONS

None.

PRESENTATIONS

None.

TABLE 1

IN VIVO MEAN PER CENT METHEMOGLOBIN LEVELS IN ARTERIAL BLOOD OF CYNO MONKEYS AFTER VARIOUS IM DOSES OF 4-DMAP AND IV $NaNO_2$

Dose	Time in Minutes 1	5	10	30	60	120
4-DMAP (mg/kg)						
4 (N = 5)	7	19	24	27	23	14
6 (N = 5)	10	28	38	46	35	20
8 (N = 5)	9	30	40	51	43	28
16 (N = 5)	12	35	49	61	52	32
$NaNO_2$ (mg/kg0						
20 (N = 5)	-	13	19	30	38	34

TABLE 2

MAXIMAL EFFECTS OF 4-DMAP (4 mg/kg) AND $NaNO_2$ (20 mg/kg) ON VARIOUS PHYSIOLOGICAL PARAMETERS IN MONKEYS

	Per Cent of Control	
Parameter	DMAP	$NaNO_2$
Respiratory Rate	No Effect	No Effect
Heart Rate	112%	123%
Blood Pressure	91% (15 min)	67% (15 min)

TASK AREA AC/WORK UNIT 026

EFFICACY OF ORGANOPHOSPHINATES AS PROPHYLACTIC AGENTS IN NERVE GAS INTOXICATION

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DACG 6508	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A826	AC	026
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)*
(U) Efficacy of Organophosphinates as Prophylactic Agents in Nerve Gas Intoxication

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
002300 Biochemistry; 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	3.5	849
	CURRENT 81	1.0	349

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 301-671-3836
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Sultan, W.E.
NAME: Lennox, W.J. POC: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Phosphinates (U) Prophylaxis (U) Nerve Agents (U) Organophosphate (U) OP Poisoning

23. TECHNICAL OBJECTIVE.* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess the potential of phosphinates as effective prophylactic agents for the solider in nerve agent poisoning, to determine their mechanism of action, and to compare their efficacy and mechanism of action with carbamate prophylaxis.

24. (U) Synthesize selected phosphinate esters and study their chemical, enzymatic, toxicological, and prophylactic characteristics.

25. (U) 80 04 - 80 09. The synthesis of twenty-six phosphinate esters has been completed. We have examined the hydrolytic stability, cholinesterase inhibition parameters, and the responsiveness of the inhibited enzyme to oximes of approximately ten phosphinate esters. Toxicological studies in mice have been completed with p-nitrophenyl dimethylphosphinate and p-nitrophenyl methyl(phenyl)phosphinate. Both compounds are less toxic than the carbamate pyridostigmine, the current prophylactic standard. Preliminary prophylactic testing in mice with the two phosphinate esters just noted suggests that phosphinate prophylaxic may be superior to carbamate prophylaxis. These results also suggest that the present mechanistic concept of carbamate and phosphinate prophylaxis may be incomplete or inaccurate. Additional animal data is urgently needed so that the toxicological and prophylactic data can be integrated with our in vitro data to assist in the design and selection of phosphinates for synthesis and testing.

*Available to contractors upon originator's approval

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PROJECT 3M162734AH26 Medical Defense Against Chemical Agents

TASK AREA AC

WORK UNIT 026 Efficacy of Organophosphinates as Prophylactic Agents in Nerve Gas Intoxication

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BACKGROUND

Conventional oxime/atropine therapy will provide modest protection against the immediate effects of many organophosphorus compounds that are used as chemical warfare agents. However, in many cases the surviving subjects would likely be at least partially incapacitated for a period of hours to days. Unfortunately, a similar condition results if one attempts to use oxime/atropine mixtures prophylactically.

To circumvent these difficulties a number of research workers have attempted to capitalize on Koster's 1946 report that the carbamate eserine protects against several LD_{50}'s of the organophosphate diisopropylfluorophosphate (DFP) subsequently administered.

The current concept of carbamate prophylaxis is quite simple. The premise is that carbamates react with cholinesterases in a way precisely analogous to the reactions of these enzymes with organophosphates. Inhibition of a portion of an animal's cholinesterases prevents complete phosphylation or inactivation upon exposure to highly toxic organophorphorus compounds.

PROGRESS

Our approach to the problem of prophylaxis has been to synthesize a variety of phosphinate esters under contract and study in-house their chemical, enzymatic, toxicological, and prophylactic properties. Representatives of the phosphinate esters we have studied are shown in figure 1.

Examples of the various properties we have characterized are:

1. Elemental Analysis

2. IR and NMR

3. Stability at Different pHs

4. Enzyme Inhibition Constants

5. Spontaneous Reactivation of Inhibited Enzymes

6. Oxime Induced Reactivation of Inhibited Enzymes

7. Toxicity

8. Detoxification by Rat Liver

9. Binding to Nicotinic and Muscarinic Receptors

10. Mutagenic Potential

The salient point here is that, unlike many drug programs, there is no single property to correlate with prophylactic efficacy at this time.

The results of our hydrolysis studies on three phosphinate esters are shown in table 1. Our in vitro enzyme inhibition studies are carried out using stopped-flow instrumentation and automated data processing. Examples of the in vitro inhibition data we have determined with our system are shown in table 2. Table 3 compares the spontaneous reactivation results of eel and bovine erythrocyte acetylcholinesterase with several of the compounds we have studied to date. It is interesting to note that in all three cases the spontaneous reactivation observed using eel acetylcholinesterase is greater than that observed with bovine erythrocyte acetylcholinesterase.

In conjunction with the in vitro investigations carried out in our laboratory, several toxicological studies have been completed along with some preliminary prophylactic testing. A great deal more animal data are needed so that the results can be integrated with our in vitro data to assist in the design and selection of phosphinates for testing and synthesis. Toxicological studies completed to date on our phosphinate esters, table 4, have shown that in each case examined thus far, these compounds are less toxic than the carbamate pyridostigmine. Pyridostigmine has an LD_{50} in mice by 1.6 mg/kg (i.m.). By the same route of administration, p-nitrophenyl dimethyl phosphinate is only one-half as toxic, and p-nitrophenyl phenyl (methyl)phosphinate is only one-third as toxic.

Preliminary prophylaxis/TAB therapy experiments have also been completed with these two compounds, using mice and the agent soman. Our rationale for their selection was that these two compounds reflected significantly different spontaneous reactivation rates in our in vitro studies. As our in vitro work also showed that both of these compounds responded to induced reactivation by 2-PAM and TMB-4, we expected to gain a handle on the significance of the spontaneous reactivation rate in efficacy studies. For comparative purposes the carbamate pyridostigmine was selected.

The first parameter determined in our study was the dose of each phosphinate, administered i.m., needed to produce depression of blood cholinesterase by approximately 40% in one-half hour. This time and level were chosen to mimic the criteria used to select a dose for pyridostigmine, the current prophylactic standard. Table 5 shows our results with p-nitrophenyl dimethylphosphinate at a dose of 0.80 mg/kg and p-nitrophenyl methly(phenyl)phosphinate at a dose of 1.25 mg/kg. These were the doses we selected to use prophylactically. They were obtained from dose response curves. Our *in vivo* results shown here paralleled our *in vitro* studies on the spontaneous reactivation of eel and bovine erythrocyte acetylcholinesterase inhibited by these compounds. That is, the methyl(phenyl)phosphinate recovers activity much more rapidly than the dimethylphosphinate. These *in vivo* results also demonstrate that both phosphinates can cross the blood-brain barrier.

Table 6 summarizes our prophylactic results to date with these two compounds. The dimethylphosphinate is significantly superior to pyridostigmine when 2 LD_{50} GD are administered i.m. The methyl(phenyl)phosphinate also appears to be better at this level.

While we find this 24-hour prophylaxis data very encouraging, the experiments did produce some surprising results. For example,

1. If a "protected" carbamylated enzyme is important, why did we observe any saves with pyridostigmine? It is generally accepted that pyridostigmine would have no beneficial effect in a 24-hour prophylactic regimen.

2. If a "protected" phosphinylated enzyme is important, how does one account for the efficacy of *p*-nitrophenyl methyl(phenyl) phosphinate? As shown on the previous slide, both the blood and brain cholinesterases completely and spontaneously reactivated in 24 hours when mice were given a prophylactic treatment with this compound.

Our results to date suggest that our present concept of carbamate and phosphinate prophylaxis is incomplete. Now that we are aware of this fact, we can design our future experiments to help identify their modes of action.

PUBLICATIONS

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PRESENTATIONS

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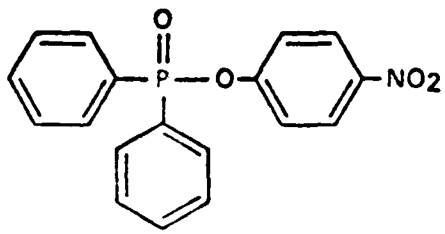
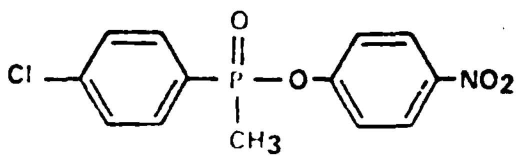
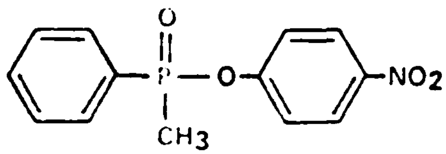
ORGANOPHOSPHINATES USED IN CURRENT STUDIES



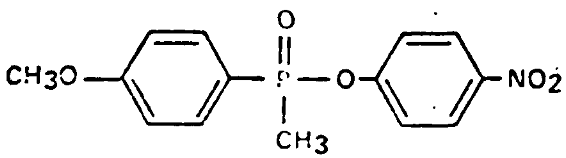
p-NITROPHENYL DIPHENYLPHOSPHINATE



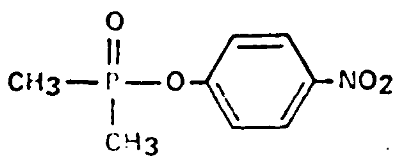
p-NITROPHENYL *p*-CHLOROPHENYL(METHYL)PHOSPHINATE



p-NITROPHENYL PHENYL(METHYL)PHOSPHINATE



p-NITROPHENYL *p*-METHOXYPHENYL(METHYL)PHOSPHINATE



p-NITROPHENYL DIMETHYLPHOSPHINATE

Figure 1

TABLE 1

HYDROLYSIS RESULTS FOR DPP[a], MPP[b], AND DMP[c] AT 25.0°C IN THREE BUFFERS.

pH	BUFFER	DPP ($t_{1/2}$ in min)	MPP ($t_{1/2}$ in min)	DMP ($t_{1/2}$ in min)
6.90	0.067 M PHOSPHATE BUFFER	158.5 (5.2)[d]	31.7 (1.1)	36.1 (0.2)
7.60	0.10 M MOPS BUFFER	211.2 (2.7)	105.0 (2.6)	278.1 (8.5)
9.10	0.10 M BICINE BUFFER	17.3 (0.4)	3.45 (0.05)	6.30 (0.02)

[a] *p* - NITROPHENYL DIPHENYLPHOSPHINATE
[b] *p* - NITROPHENYL METHYL(PHENYL)PHOSPHINATE
[c] *p* - NITROPHENYL DIMETHYLPHOSPHINATE
[d] STANDARD DEVIATION

TABLE 2

INHIBITION OF CHOLINESTERASES

INHIBITOR	ENZYME	$K_d \times 10^5$ (M)	$k_2 \times 10$ (SEC^{-1})	$k_i \times 10^{-2}$ (M^{-1} SEC^{-1})
DMP[b]	Eel AChE	190. (32.)[a]	6.19 (1.45)	3.23 (0.23)
	BVE AChE	144. (12.)	5.71 (0.51)	3.98 (0.04)
	BuChE	147. (12.)	4.91 (0.46)	3.34 (0.05)
MPP[c]	Eel AChE	0.730 (0.084)	2.11 (0.33)	289. (14.)
	BVE AChE	20.0 (7.8)	16.2 (6.2)	80.9 (0.8)
	BuChE	7.60 (2.28)	23.3 (6.3)	308. (5.)
DPP[d]	Eel AChE	61.7 (12.1)	0.0960 (0.0462)	0.150 (0.048)
	BVE AChE	142. (6.)	1.62 (0.15)	1.14 (0.07)
	BuChE	2.96 (0.11)	0.270 (0.012)	9.13 (0.15)

[a]STANDARD DEVIATION

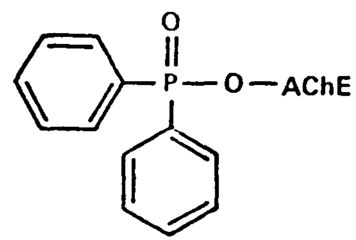
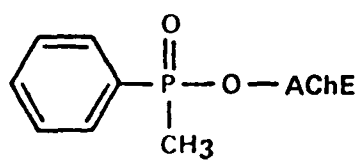
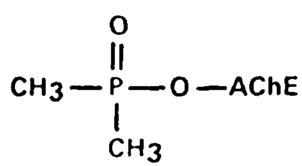
[b]p-NITROPHENYL DIMETHYLPHOSPHINATE

[c]p-NITROPHENYL METHYLPHENYLPHOSPHINATE

[d]p-NITROPHENYL DIPHENYLPHOSPHINATE

TABLE 3

SPONTANEOUS REACTIVATION OF INHIBITED CHOLINESTERASES, 0.10 M MOPS BUFFER, pH 7.60, 25.0°

INHIBITED SPECIES	% SR IN 24 HOURS EEL AChE	% SR IN 24 HOURS BVE AChE
	9.1 (2.3)*	0.94 (0.04)
	41.8 (1.6)	12.3 (0.2)
	2.0 (0.8)	0.31 (0.06)

*STANDARD DEVIATION

TABLE 4

TOXICITY OF PHOSPHINATES IN MICE

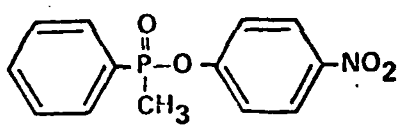
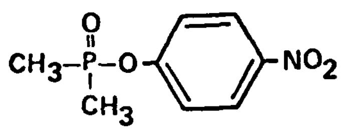
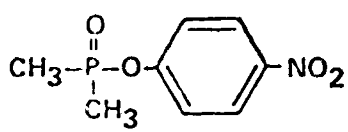
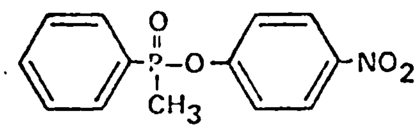
Compound	LD50 (im) (mg/Kg)	LD50 (oral) (mg/Kg)
	*ca.* 5.0	6.7 (5.8-7.9)
	4.0 (3.2-5.0)	69 (59-80)

TABLE 5

CHOLINESTERASE LEVELS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF p-NITROPHENYL DIMETHYLPHOSPHINATE (1/5 LD50) AND p-NITROPHENYL PHENYL(METHYL)PHOSPHINATE (1/4 LD50)





TIME (HRS)	% INHIBITION		TIME (HRS)	% INHIBITION	
	BLOOD ChE	BRAIN ChE		BLOOD ChE	BRAIN ChE
0.5	45	27	0.5	40	64
1.0	47	27	1.0	37	59
3.0	57	33	3.0	29	63
24	28	31	24	0	2

TABLE 6

24-HOUR PROPHYLAXIS RESULTS FOR $2LD_{50}s$ OF SOMAN GIVEN IM (MICE)

COMPOUND	THERAPY TIME[a]	
	10 SECONDS	30 SECONDS
p-NITROPHENYL DIMETHYLPHOSPHINATE	10[b]	10
p-NITROPHENYL METHYL(PHENYL)PHOSPHINATE	8	8
PYRIDOSTIGMINE	5	4

[a] TAB

[b] NUMBER OF SURVIVORS OUT OF 10 ANIMALS

TASK AREA AC/WORK UNIT 030

ANALYSIS FOR POTENTIAL TOXIC MATERIAL(S)
IN AGED ATROPEN INJECTOR

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*		REPORT CONTROL SYMBOL
				DA0G6492	80 04 09		DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734AH26	AC	030
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*
(U) Analyses for Potential Toxic Material(s) in Aged Atropen Injector

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
002300 Biochemistry; 008300 Inorganic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.:

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	0.0	00
	CURRENT 80	3.2	620

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Biomedical Laboratory
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION
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ADDRESS:* Aberdeen Proving Ground, MD 21010
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Ellin, R.I.
TELEPHONE: 301-671-2206
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Kaminskis, A.
NAME: Sultan, W. POC: DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Atropine, (U) Antidote, (U) Automatic Injector, (U) Potency, (U) Storage, (U) Stability, (U) Toxic Component(s)

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Aged Atropine Sulphate Injectors for Nerve Agents Antidote (Atropine Injector) produce lethality when injected into mice at a dose proven safe on produce acceptable tests. The objectives of this effort are to determine the cause of the toxicity in aged injectors, develop a precise analytical method for assay of the toxic component(s), and develop analytical criteria to establish quality standards for the retention of Atropine Injectors in inventory based on safety for the toxic component(s).

24. (U) Separation, isolation and quantitation will be performed by liquid, chromatography, atomic absorption, and mass spectrometry. Additional analytic techniques include electron microscopy, electron probe microanalysis. Toxicity evaluations will be determined by the purchase specification i.p. mouse toxicity and the i.v. mouse toxicity delivered at USABML.

25. (U) 8004-8009. On 9 June 1980, USABML reported that zinc ion leaching from the rubber on the cartridge was the cause of the toxicity of aged Atropen injectors. Cartridges that contained greater than 0.5 milligrams per ml of zinc were toxic to mice, less than 0.4 milligrams per ml of zinc were non-toxic. Toxicity is attributed as either zinc sulfate, zinc citrate or zinc salts. Zinc sulfate, zinc citrate, or zinc metal, when added to an atropen formula, produced identical results. In addition, it was determined that the zinc content within the rubbers in both the aged toxic and aged non-toxic lots cartridges were originally identical. Consequently, the zinc from the rubber of the lots leached more readily into the atropen injection solution than the zinc in the rubber non-toxic lots.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M162734AH26 Medical Defense Against Chemical Agents

TASK AREA AC Analyses for Potential Toxic Material(s) in Aged Atropen Injector

WORK UNIT 030

INVESTIGATORS R.I. Ellin
A. Kaminskis
W. Sultan

BACKGROUND

At the request of the Commander, US Army Medical Research and Development Command, the US Army Biomedical Laboratory undertook studies to find out why the atropen injector became toxic and to identify the toxic component(s) in aged atropen injectors.

The vendor who supplied the atropen injectors containing atropine sulfate injection (for nerve agent antidote) had originally reported that the oldest retained samples produced lethality when injected into mice at a volume that was originally proven safe on product acceptance tests. Atropen injectors were obtained from Army inventory lots without documented storage histories. Selected samples of these return lots were tested in FDA laboratories. The results of these tests also confirmed that the oldest lot proved to be toxic in mice.

PROGRESS

A zinc compound present in rubber enclosures is primarily the cause of the toxicity of aged atropen injectors. Atropens obtained from the field that contained 0.5 milligrams of zinc per ml or more in an injector were toxic when evaluated by the mouse-safety test. Atropen cartridges that contained 0.4 milligrams of zinc per ml or less were not toxic. This data was corroborated in separate experiments when concentrations of zinc either as zinc citrate, zinc sulfate or as zinc metal were added to freshly prepared contents of the atropen cartridge. (Therefore the toxicity results from the zinc as zinc citrate plus any other zinc salt present.) Atropine formulations which contained greater than 0.5 milligrams of zinc were found toxic; these with 0.4 milligrams or less were non-toxic. Lots obtained from the field and even similar lots, which had been stored and retained at room temperatures by the manufacturer of Atropens (Survival Technology), had identical toxicity on the basis of zinc content.

The calculated zinc content of the rubber enclosures of toxic and non-toxic lots are identical. Consequently the zinc compound in rubber leached more readily from the rubber enclosures in the toxic Atropens than from the non-toxic Atropens.

Atropens that are toxic have significantly higher pH values than non-toxic Atropens. This may be caused by zinc compound from rubber reacting with citrate buffer in the Atropen formulation. The higher pH would cause more rapid decay of an Atropine. Toxic Atropens consistently show lower atropine concentrations than those that are non-toxic.

The zinc content in toxic lots of Atropens was 0.4% of the weight of the rubbers used. Rubber inclosures, both plunger and stopper, presently used in the manufacture of Atropens were recently assayed to contain 0.1% zinc or less. By rigid control of the zinc content of rubber, not only would the formulation be non-toxic, but atropine would be more stable. The shelf-life of the Atropen Injector could be extended from five to ten years.

A quantitative mouse test applying intravenous administration was developed to determine toxicities of Atropens. Slopes are steep. Results for toxic lots were 3.4 ml/kgm, non-toxic lots 8.7 ml/kgm, reference standard, 13 ml/kgm.

RECOMMENDATIONS

The recommendations presented here are: for the purpose of correcting the defect in Atropens which caused this situation, to assure that such situations do not arise in the future, to develop a product improvement program which will extend the shelf of existing injectors as well as assist USAMRDC in rapid, efficient and reliable product development in the future.

1. The results presented in this document clearly demonstrate that the cause for toxicity development in aged Atropen is that in toxic lots sufficient quantity of zinc in the form of a zinc compound leaches from rubber components of injectors into the Atropen solution. Zinc in the form of zinc citrate and zinc salt are lethal to mice in concentrations of zinc which can be defined as greater than 0.5 mg/ml. By changing the formulation, the zinc toxicity problem might be resolved. On the other hand, other problems and questions could arise: what new solvents(s) should be used?, how stable are the ingredients in the Atropen in this solvent?, would the new solvent cause problems in toxicity?, and most significantly, might not a new formulation require a new NDA?

2. In order to prevent zinc concentrations from forming in Atropens at non-toxic levels, it is recommended that the following specifications be included in future purchase contracts: "The zinc content of all rubber inclosures used in the Injector item will not exceed 0.1% of the weight of the injector." The reason for establishing this value is that over a 13 year period, 26% of the zinc leached from rubber enclosures in toxic lots, 6% from non-toxic lots. The inclosures weigh about 0.5 gram each or a total of 1 gram. One-tenth per cent of total rubber is 1.0 milligram. If one takes into consideration the results of toxic lots, the amount of zinc per cartridge over a 13 year period would be a total of 0.26 milligrams. This concentration is non-toxic in the mouse safety test.

It would be ideal to write a specification for zinc in a rubber which, even upon total leaching, would not provide enough zinc to be toxic to mice. In this case we would recommend not more than 0.1 milligram per rubber item in the injector. At this time we don't know the feasibility of such a recommendation.

3. The present shelf-life of Atropens has been set at five years from date of manufacture. Since more is now known about Atropen stability and toxicity as a result of these investigations, the following recommendations will (a) permit establishment of firm shelf-life, (b) assure that at all times the product in the field is of perfect biomedical quality and (c) provide tangible financial benefit at time of replacement, since there is sufficient evidence to support a shelf-life of the Atropine formulation of greater than five years. It is recommended that upon purchase of Atropens, one hundred injectors from each lot be sent to USABML to perform the following tests every six months in addition to the ones required by FDA and DPSC: (a) determine the zinc content of the formulation, (b) perform the mouse toxicity assay. The data generated from such tests will facilitate the request to FDA for extension of shelf-life of field injectors.

4. A product improvement and development program should be established. The nerve agent antidote formulations, unlike other biomedical formulations, have a unique requirement. The biomedical formulations are designed for continuous use as needed; thus turnover of lots will eliminate the need for long storage. The cost of storage, accountability, and replacement when they become outdated is therefore substantial. In addition, the usefulness of antidotes is for protecting the military, who at the time of use, is located in a strategic position. Of prime importance is assurance that they retain their potency and are safe to use at any time. Under usual circumstances FDA regulations and the manufacturer's testing, in conjunction with DPSC, will meet this requirement. However, it leaves little latitude for changes or improvement of the product.

5. The shelf-life of Atropens may be extended for an additional five years. Cost savings were discussed with value-engineering coordinators at DPSC. The following three areas were considered: (1) immediate stock on hand that is five years old but under ten years old, (2) planned procurement over the next five years based on current demand data, (3) current stock on hand, less than five years old. At the present price of $3.25 per injector, DPSC estimated a cost savings of 20.3 million dollars.

6. There are additional areas of investigations which can be carried out in-house to improve quality of products. (a) Develop effective antidote formulations useful under extreme climatic conditions, (b) develop a test for material leached from rubber that would ascertain the safety of an injector item, (c) develop a practical animal safety test that would quantitatively define toxicity, (d) study stability under a variety of storage conditions, (e) study compatability of mixtures of drugs in various formulations, (f) study solubilities of drugs in solvent vehicles to determine the possible dosage formulations, (g) project and study novel dosage forms for potential use in the field, and (h) study the rate and extent of absorption and distribution of formulations in tissues.

7. It is recommended that a requirement be established for product improvement and development. Investigations can be carried out jointly by USABML and WRAIR. Since it has been shown that such programs will result in substantial savings in the long run, appropriate funding will result in further monetary, time and effort savings.

PRESENTATIONS

None.

PUBLICATIONS

None.

REFERENCES

Atomic Absorption Analyses for Zinc and Metals were performed under contract with Product Assurance Directorate, Chemical Test Branch, USARRADCOM Support Element, Aberdeen Proving Ground, MD 21010.

PROJECT 3S162772A875

MEDICAL SYSTEMS IN NON-CONVENTIONAL ENVIRONMENTS

TASK AREA BA/WORK UNIT 201

BEHAVIORAL TOXICOLOGY OF NERVE AGENTS AND TREATMENT
WITH PROPHYLACTIC AND THERAPEUTIC COMPOUNDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY		1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
		DAOG 6513	80 10 01	DD DR&E AR 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 08	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	BA	201
b. CONTRIBUTING				
c. CONTRIBUTING		STOG 80-7.2:1		

11. TITLE (Precede with Security Classification Code)* (U) Behavioral Toxicology of Nerve Agents and Treatment with Prophylactic and Therapeutic Compounds.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 016800 Toxicology; 012600 Pharmacology; 012900 Physiology; 013400 Psychological

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER*

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	6.5	1430
	CURRENT 81	3.5	596

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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TELEPHONE: 301-671-2373

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Penetar, D.M.

NAME: King, J.M.

POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) CW agents (U) Anticholinesterase (U) Anticholinergic (U) Animal behavior

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define, develop, and validate a series of animal behavioral tests to serve as models which reflect the neurobiological effects of CW agents and how these effects are mitigated by prophylaxis/therapeutic compounds. Included in development and validation of these models is a rationale for generalization of results to man.

24. (U) Animal behavioral tests are used to assess physiological and psychological function following three types of experimental treatments: CW agent alone, prophylaxis and/or therapy alone, and CW agent exposure combined with prophylaxis and/or therapy. Studies are conducted with acute high dose and chronic low dose exposures to CW agents.

25. (U) 8004-8009. Three tests have been developed and validated which reflect motor and behavioral incapacitation following sub-lethal exposure to Soman. Prophylactic and therapeutic mixtures have been tested for ability to reverse incapacitation/lethality. Therapeutic mixture reverses lethality, and to an extent, motor incapacitation; prophylactic mixture reverses all effects. Dose-response studies to establish behavioral effects of 2-PAM in rodents have been initiated. Dose-response studies of anticholinergic drugs on nonhuman primate learning and memory are continuing. Interaction studies between morphine, anticholinergics, anticholinesterase, and stress on pain perception are continuing. Four research papers from this work have been presented in 1980: 3 Society for Neuroscience, 1 American Society of Pharmacology and Experimental Therapeutics.

*Available to contractors upon originator's approval

DD FORM 1498 (MAR 68)

PROJECT 3S162772A875 Medical Systems in Non-Conventional Environments

TASK AREA 3A

WORK UNIT 201 Behavioral Toxicology of Nerve Agents and Treatment with Prophylactic and Therapeutic Compounds

INVESTIGATOR(S) J.H. McDonough
J.M. King
D.M. Penetar
J.A. Romano

BACKGROUND

Behavioral responses to toxic agents are many times more sensitive indicators of toxic chemical insults than measures such as lethality. Use of behavioral responses as indices of toxicity provides planners with data on risk vs. benefit trade-off between unwanted side-effects and prophylactic/therapeutic efficacy of drugs under consideration. A variety of behavioral tests are used in rodents and subhuman primates to assess the integrity of motor behavior, sensory function, homeostatic behavior (feeding, drinking, body weight regulation, thermoregulation), motivation, and cognitive function. Dose-response functions for descriptions of these normal behaviors are determined under acute, sublethal challenge with nerve agent or after treatment with prophylactic/therapeutic compounds, either alone or in combination with agents. In this testing program other carbamate or organophosphate compounds are used to establish the validity and generality of the behavioral tests.

PROGRESS

All thirteen (13) protocols in this group were active in FY80. Substantial progress was made in some protocols; others were established with parametric studies being undertaken.

Effects of Various Prophylactic and/or Therapy Compounds on Responses to Noxious Stimulation.

This program includes three protocols. Work completed in 1980 indicates that carbamate anticholinesterases will impair an organism's ability to respond to noxious stimulation. This impairment is due, at least partially, to the invocation of a generalized stress response with neuroendocine correlates and such impairment may be reversed by anticholinergic drugs such as benactyzine HCl. This impairment is also related to an opiate response and shows interactions with opiate agonists. The organism's lessened ability to respond to noxious stimuli reflects changes in both the sensory or discriminative aspect of the pain sensation and in the affective (emotional) response to the noxious stimulation. Lastly, it was shown that this dual response system may be manipulated so as to selectively affect one or both of the responses to painful stimuli.

Feeding, Drinking and Taste Aversion Studies.

Studies of effects of centrally-acting versus peripherally-acting anticholinergic drugs on drinking behavior were performed in FY80. Atropine sulfate and atropine methyl nitrate were found to suppress water intake equally well in rats. Benactyzine HCL was essentially without effect.

The conditioned taste aversion (CTA) protocol was approved in the 2d quarter of FY80. CTA studies were begun in the 3d quarter. Initial work established undrugged concentration preferences for saccharin and saline. Subsequently, a dose-response relationship was established for a reference substance, lithium chloride. Significant advancement is expected to be achieved in FY81.

Effects of Various Agents, Prophylactic and/or Therapy Compounds on Thermoregulation.

Data from the investigation will provide valuable information on the ability of individuals who were exposed to various compounds of interest to cope with thermal stressors. Work was initiated on this protocol in FY80.

Drug Discrimination Procedures.

Animals were trained to emit a particular response under one set of conditions (drug) while under a second set a different response is appropriate. The results of these experiments in FY80 have shown that animals are capable of discriminating both carbamate and organophosphorous (Soman) cues from saline and other drug cues. Furthermore, it was shown that the discriminable cues produced by Soman persist for up to 30 hours post-injection.

Behavioral Deficits Produced by Therapeutic Compounds on Short-Term Memory, Time Perception and Learning Ability.

Rhesus monkeys are being tested under a behavioral paradigm that requires them to remember a sample color for up to 16 seconds and then correctly match it in order to earn a food reward. Two anticholinergic drugs - atropine and benactyzine and the antidote mixture TAB have been tested in a dose-response fashion. These same drugs have been tested in two other behavioral paradigms - one which requires rhesus monkeys to accurately judge a span of 28 seconds before responding in order to earn a food reward and a second which requires cynomolgus monkeys to learn a sequence of correct responses (4 component sequence) in order to earn a reward.

Results from all three behavioral measures have been similar. Atropine at 0.014 mg/kg produces little or no disruption; atropine at 0.44 mg/kg produces profound and prolonged (up to 8 hrs) behavioral disruption with graded effects at doses between these extremes. Benactyzine at 0.057 mg/kg produces little or no disruption while 1.82 mg/kg produces a severe but of short duration (<30 minutes) behavioral deficit. TAB, at a monkey dose equivalent to one Combopen®, produces some reliable but probably not statistically significant behavioral effects.

Prophylactic Drug Administration on Motor Behavior.

Pyridostigmine at a dose which produces 50% cholinesterase inhibition has been tested in cynomolgus monkeys performing under an increasing work output schedule. When chronically administered over 3 weeks, pyridostigmine has been found to decrease work output ability by one-third to one-half of baseline performance. Animal's performance recovers within 3 days after drug administration ceases.

Shuttle Avoidance Procedures.

Behavioral intoxication and physical incapacitation produced by Soman have been studied using 2-way shuttle avoidance tests. Animals were trained to avoid shock by "shuttling" to another compartment when a warning stimulus appeared. Results indicated that at lower doses (20, 30, 40 ug/kg) animals were capable of performing escape responses; at higher doses animals failed to escape or avoid shock. This dose dependent decrement of performance was reversed by TAB, with benactyzine the component of TAB which provided the greatest protection against the incapacitating effects of Soman.

Collaborative Ventures with other Agencies.

During the last month of FY80 the Behavioral Toxicology Branch undertook a large scale investigation in collaboration with the Division of Neuropsychiatry, WRAIR, to study effects of circadian shifts in Ach levels on Soman Lethality, and long term effects of acute treatment with Soman on body weight, lethality, and responses to noxious stimuli. Range finding studies were completed in FY80, the major project to be completed in FY81.

PUBLICATIONS

King, J.M. & Cox V.C. Relationship Between Body Weight and Estradiol Induced Activity Physiology and Behavior. 24 (4), 657-659 (1980).

PRESENTATIONS

Harris, L., et al. Protection Against Both the Lethal and Behavioral Effects of Soman. The Pharmacologist. 22: 239 (1980) presented at the ASPET Meeting, Rochester, MN, August 1980.

Three abstracts were accepted by the Society for Neurosciences. They were:

King, J.M. & Romano, J. Enhancement of Physostigmine Analgesia by Morphine: Dependence on Dose and Test System. Soc. Neurosci. Abstr. 1980, 6, 149.18.

Penetar, D. Anticholinergic and Anticholinesterase Effects on a Repeated Acquisition Baseline. Soc. Neurosci. Abstr. 1980, 6, 383.

Romano, J. & King, J.M. Benactyzine-Induced Reversal of Physostigmine and Cold-Water Analgesia. Soc. Neurosci. Abstr. 1980, 6, 149.17.

Also the following abstracts have been prepared and were accepted for presentation:

McDonough, J., Hackley, B., Cross, R., Sampson, F. & Nelson, S. Brain Regional Glucose Use During Soman-Induced Seizures. Accepted for publication at FASEB meeting, April 1981.

McDonough, J., Penetar, D., Jackson, J. & Zimmer, G. Behavioral Effects of Soman in Rats and Modification of These Effects with Prophylactic or Therapeautic Compounds. TTCP presentation, October 1980, Washington, DC.

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TASK AREA BF/WORK UNIT 202

PHYSIOLOGICAL CONSEQUENCES OF NERVE AGENT EXPOSURE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
	DA0G6512	80 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	BF	202
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Physiological Consequences of Nerve Agent Exposure

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	1.5	152
	CURRENT 81	3.7	460

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Biomedical Laboratory

ADDRESS: Aberdeen Proving Ground, MD 21010

RESPONSIBLE INDIVIDUAL

NAME: Llewellyn, C.H.

TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION

NAME:* US Army Biomedical Laboratory

ADDRESS:* Aberdeen Proving Ground, MD 21010

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Rickett, D.L.

TELEPHONE: 301-671-2373

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

PII Redacted

21. GENERAL USE

Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS

NAME: Glenn, J.F.

NAME: Beasley, D.E. POC:DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Electrophysiology (U) CNS (U) Audition (U) Respiration (U) Sleep (U) Sensory systems (U) Information Processing (U) P&T Compounds

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Identify physiological, including neurophysiological, mechanisms of action of nerve agents and P&T compounds both centrally and peripherally to include duration of action and dose-response relationships.

24. (U) Investigate mechanisms of nerve agent produced respiratory arrest, persistant sleep/wake and arousal threshold deficits, alterations in attention and sensory information processing using electrophysiological techniques. The efficacy of putative P&T regimens in prevention or treatment of observed deficits will be evaluated.

25. (U) Soman produced respiratory arrest is attributable to disruptions of CNS respiratory drive mechanisms prior to the appearance of peripheral neuromuscular blockade. Data collection is in progress for assessment of soman's effects of sleep/wake activity and arousal thresholds. Equipment is being purchased to support identification and treatment of soman produced alterations in attention and sensory information processing.

*Available to contractors upon originator's approval

DD FORM 1498, 1 MAR 68 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

PROJECT 3S162772A875 Medical Systems in Non-Conventional Environments

TASK AREA BF

WORK UNIT 2(Physiological Consequences of Nerve Agent Exposure

INVESTIGATORS Rickett, D.L.
Glenn, J.F.
Beasley, D.E.

BACKGROUND

Mechanisms of Respiratory Arrest.

The primary cause of death from acute exposure to lethal concentrations of organophosphorus (OP) chemical warfare (CW) agents is generally conceded to be cessation of respiration. Respiratory arrest may be mediated by both peripheral and central events manifested as airway obstruction by salivary and bronchial glandular secretions, laryngospasm, bronchoconstriction, neuromuscular blockade of the muscles of respiration and arrest of normal activity within those areas of the central nervous system (CNS) which control respiratory function (Brimblecombe, 1977; Wolthuis, 1976). Although it is technically feasible to protect against the lethal effects of CW threat agents, this protection is offered at the unacceptable cost of prolonged incapacitation of individuals receiving treatment. This is true, regardless of whether they were actually exposed to agent or to the extent of agent exposure. Absolutely essential to development of a maximally effective and a minimally debilitating prophylaxis and therapy regimen is identification and understanding of the sites and mechanisms of action of these agents, as well as the relative contributions of these actions, in the generation of lethal effects. This information must be developed for both acute and subacute exposure. It is also important to develop a knowledge of immediate and persistant functional deficits which ensue as a consequence of acute or subacute exposure to sublethal concentrations of agent, or survival of a lethal challenge.

Effects of Cholinoactive Compounds on Sleep-Wake Behavior.

The symptomology of individuals exposed to various anticholinesterases (anti-ChE) including soman (Sidell, 1974) and sarin (Grob, 1956; Grob, Harvey, Langworthy, & Lilienthal, 1947) include: excessive dreaming, insomnia, memory impairment, mental confusion, visual hallucinations, fatigue and trouble concentrating. These symptoms are strikingly similar to behavioral deficits reported following either partial or total sleep deprivation and may persist for periods of weeks to several months following otherwise complete symptomatic recovery (Sim, 1965). If these kinds of symptoms are common to troops who survive an exposure to anti-ChE CW agents, their tactical mission capabilities would be seriously jeopardized. Clearly, the effects of these agents on sleep and arousal should be investigated in order to provide a commander with information concerning the capabilities of exposed troops, as well as, to provide medical personnel with information critical to return-to-duty criteria. This is true whether the exposure which was treated was subacute low-dose, acute low-dose, or acute lethal. Additionally, this research is necessary for planning the extent and kinds of medical treatment that exposed troops might require.

PROGRESS

Mechanisms of Respiratory Arrest.

During the past year, our research focused on differentiation of central and peripheral mechanisms of action of soman in the production of respiratory arrest. The results of this effort which are being presented at the 1980 Meeting of the Society for Neuroscience, clearly showed that the cause of respiratory arrest was a loss of central drive attributable to a loss of synchronized firing of respiratory-related neurons in the brainstem. This established the need for centrally active treatment compounds. It also resulted in the development of a model system useful for testing efficacy of future P&T compounds in reversing identified physiological components of respiratory arrest including: activity of respiratory-related units in the brainstem, phrenic nerve discharge, diaphragmatic contraction and electromyrogram (EMG), airflow, blood pressure and cardiographic measurement.

Effects of Sublethal Exposure to Soman on Sleep Wake Cycles and Arousal Thresholds.

Last year we also started collecting data on the effects of an acute, sublethal exposure to soman on sleep-wake cycles and arousal thresholds. Using cats, we have thus far seen that post-exposure sleep patterns are disrupted for at least 9 months and that arousal thresholds are elevated. While these results are preliminary, they suggest a need for long-term treatment of individuals following a single exposure to nerve agents.

PUBLICATIONS

None.

PRESENTATIONS

An abstract entitled "Differentiation of Central and Peripheral Actions in Soman-Induced Respiratory Arrest" by D.L. Rickett, N.L. Adams, K.J. Gall, S.F. Rybczynski, T.C. Randolph, has been accepted for presentation at Society for Neuroscience Annual Meeting, 11-14 November 1980 at Cincinnati, Ohio.

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PROJECT 3M161102BS10

RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

TASK AREA EC/WORK UNIT 381

MECHANISM OF ACTION OF ANTICHOLINESTERASES
AND ANTICHOLINESTERASE ANTIDOTES

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
	DAOG 6519	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY		3M161102BS10	EC	381
b. CONTRIBUTING	61102A			
c. CONTRIBUTING		STOG 80-7.2:1		

11. TITLE (Precede with Security Classification Code)*

(U) Mechanism of Action of Anticholinesterases and Anticholinesterase Antidotes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012600 Pharmacology; 01680 Toxicology; 0023 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	Cont	DA	C. In-House

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	1.5	178
	CURRENT 81	5.6	320

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Biomedical Laboratory

ADDRESS:* Aberdeen Proving Ground, MD 21010

RESPONSIBLE INDIVIDUAL

NAME: Llewellyn, C.H.

TELEPHONE: 301-671-3276

20. PERFORMING ORGANIZATION

NAME:* US Army Biomedical Laboratory

ADDRESS:* Aberdeen Proving Ground, MD 21010

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Lenz, D.E.

TELEPHONE: 301-671-3074

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Broomfield, C.E

NAME: Maxwell, D. POC:DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Acetylcholine receptor, (U) Acetylcholinesterase, (U) Phosphinates, (U) Chronic Poisoning, (U) Isoenzymes, (U) Spin labels

23. TECHNICAL OBJECTIVE.* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) It is the mission of USA Biomedical Laboratory to develop a comprehensive approach to prophylaxis and therapy as a means of protection against organophosphorus compounds. This research plan is designed to provide a sufficiently broad data base upon which such a comprehensive approach can be developed.

24. (U) Succinctly stated, to investigate the mechanism of action of anticholinesterases and anticholinesterase antidotes and their relationship to enzymes, neurotransmitters hormones, receptors and membrane function with regard to cholinergic and non-cholinergic systems.

25. (U) a) The structure of spin-labelled membrane lipids from Torpedo ray was not changed upon reaction with nicotinic agonists and antagonists. Among several compounds tested as potential agonists, antagonists, or allosteric affectors of acetylcholine receptors, only a few bisquaternary oximes were bound to the nicotinic receptors, with ED50 values of 2-6 x 10E-05M. Muscarinic receptors bound most of the bisquaternary compounds weakly (ED50 higher that 10E-04M). Two bisquaternary compounds, HH64 and SAD 128 were strongly bound. b). In rats dosed with one LD50 soman all four isoenzymes of acetylcholinesterase were inhibited by soman, but some were more resistant than others. c). In rats chronically dosed with 0.5 LD50 soman for up to six weeks, the acetylcholinesterase isoenzyme inhibition profile was the same as an acute dosing inhibition profile. The acetylcholine levels were elevated in two brain areas after two weeks, but subsequently returned to normal. A reduced number of acetylcholine receptors in the diaphragm were revealed by radiolabelled I-bungarotoxin after six weeks. d).In the presence of probes sensitive to allosteric effects of changes in membrane fluidity, solubilized and membrane bound acetylcholinesterases exhibited altered kinetic behavior toward paraoxon. e) Established techniques and initiated investigation on the effects of soman on an isolated adrenal cell assay.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10 Research on Military Disease, Injury and Health Hazards

TASK AREA EC

WORK UNIT 381 Mechanism of Action of Anticholinesterases and Anticholinesterase Antidotes

INVESTIGATORS David E. Lenz, Ph.D.
Donald M. Maxwell
Clarence A. Broomfield, Ph.D.

BACKGROUND

In spite of 35 years of effort, the pharmacology of anticholinesterase compounds, which may be used in warfare situations, has not been unambiguously elucidated. The enzyme acetylcholinesterase (AChE) is usually declared to be "the" primary site of action. Unfortunately, a good correlation between degree of enzyme inhibition and toxicity is lacking. Furthermore, the importance of the *in vivo* locale of the enzyme, i.e., central or peripheral, is the subject of considerable controversy. The situation is further compounded by a lack of information regarding the *in vivo* distribution of the organophosphorus compounds, the susceptibility of other physiologically important enzymes to inhibition by organophosphorus compounds, and the effect of increased acetylcholine levels on its receptor. This deficit of information precludes the development of a model capable of explaining the existing body of knowledge, or capable of being used to design, in a rational,well understood fashion, new compounds for use in treatment or in prophylactic regimens.

PROGRESS

The role of AChE has been explored in two avenues. Firstly, the differences in the kinetic properties of the various isoenzymes of AChE were investigated. Secondly, the kinetic properties of solubilized and membrane bound AChE were studied to identify similarities or differences inherent in the enzyme in each of these locales.

Enzyme Interactions

The susceptibility of AChE isoenzymes isolated from rat cerebrum to inhibition by soman was studied. Tissue from rat cerebrum was homogenized and four forms of the enzyme with isoelectric points (PI's) between pH 4-5 were separated by iso-electric focusing in polyacrylamide gels. Iso-OMPA (a specific inhibitor of buterylcholinesterase) failed to reduce enzymatic activity, while BW284C51 (a specific inhibitor of AChE) abolished it completely, indicating that all the enzymatic activity was due to AChE. When tissue was taken from animals sacrificed after treatment with 90 ug/kg of soman intramuscularly, it was found that there were two classes of isoenzymes which were inhibited by soman at different rates, although all forms of AChE were completely inhibited 15 minutes after treatment. Currently studies are underway to examine the generality of these results for other organophosphorus compounds, such as sarin or diisopropylflurophosphate (DFP),and for carbamate such as moban or physostigmine.

Using either membrane-bound ($AChE_M$) or solubilized ($AChE_S$) forms of acetylcholinesterase from electric eel, similar kinetics were observed in the absence of inhibitor or in the presence of tensilon or trimethylammonium ion: $K_m = 1.1 \pm .1 \times 10^{-4}M^{-1}$, $K_i = 7.3 \pm .2 \times 10^{-7}M^{-1}$ and $K_i = 3.0 \pm .1 \times 10^{-3}M^{-1}$, respectively. Using paraoxon, no difference was observed between the percent inhibition at any given concentration or the concentration at which inhibition was first observed.

In the presence of F^- (Domenech et al. (1977) FEBS Lett. 74, 243-246) the relative rate of $AChE_M$ was reduced more rapidly than $AChE_S$, whether or not paraoxon was present. When paraoxon inhibition was studied in the presence of F^-, $AChE_S$ had a Hill coefficient of 1.0 at 10^{-7}-10^{-4}M paraoxon, whereas the value of $AChE_M$ changed from 0.8 at 10^{-7}-10^{-5}M to 1.6 at 10^{-4}M paraoxon. When examined in the absence of F^-, $AChE_M$ and $AChE_S$ appear to behave similarly toward various inhibitors. However, in the presence of a probe sensitive to allosteric effects or changes in membrane fluidity, the two forms of AChE exhibit altered behavior toward paraoxon. This project is currently in abeyance, pending further results from Dr. Broomfield's investigations of receptor membrane structure.

Sites of Poisoning

Preliminary work on identifying sites of action of organophosphorus compounds has resulted in the development of a model for evaluating the actions of drugs with highly characterized pharmacological sites of action.

Extrapolation of antidote protection data from animal models to human subjects is a major pharmacological problem. The difficulty arises from the variability in antidote "Protective Ratios" observed in different species. This variability has been attributed by several investigators to "species variation." The existing data for agent toxicity in naive animals (LD50), agent toxicity in antidote-treated animals ($LD50_T$), and Protective Ratios ($LD50_T$/LD50) for all available species were analyzed. Two types of graphs were used to analyze the toxicity and anitdote protection data. An "Antidote Protection" graph plots LD50 vs. $LD50_T$ vs. mean body weight of each species and defines the species variation of agent toxicity and antidote protection. "Antidote Protection" graphs were found to be linear, allowing antidote protection to be defined by the slope and intercept of the line. Oximes, carbamates, and anticholinergics each produced distinctive patterns of effect on these two parameters. "Species Extrapolation" graphs were found to be hyperbolic, allowing toxicity and protection to be defined by a constant term and an order term. The effects of route of agent administration, type of agent, and antidote protection on these two terms were analyzed.

Therapeautic Drug Interactions

In addition to studies on AChE and the development of a model for evaluating drug or agent actions, progress has been made with regard to the role of ACh and its interaction with its receptor.

Acetylcholine receptor-rich membranes having a receptor density of about 1.5 nmoles per mg were prepared from the electroplax of Torpedo Californica by density gradient centrifugation and then labeled with a series of spin labels including derivatives of fatty acids and their methyl esters. ESR spectra were recorded in the absence or in the presence of *carbamyl choline*, *d-tubocurarine*, hexamethonium, or the organophosphorus anticholinesterase, soman. For those samples in which the spin label was highly immobilized, both conventional and saturation transfer spectra were run. Under the conditions of temperature (approximately 23^{o}) and concentrations (between 10^{-6} and 10^{-2}M) studied so far, we have seen no indication of significant changes in the structure of the bulk of the lipid in nerve-ending membranes upon reaction with agonists, antagonists, or soman. This observation is interpreted to indicate that the membrane lipids are passive in the change of permeability of the membranes during synaptic transmission, or else lipid structural changes are severely limited to the immediate environment of the protein.

We are continuing studies to see whether carbamates and organophosphorus compounds inhibit different acetylcholinesterase isoenzymes. Also muscarinic and nicotinic acetylcholine receptor levels in brain and diaphragm and muscle will be measured.

PUBLICATIONS

A chapter entitled "Antibody Quantitation Using the Unlabeled Antibody Enzyme Immuno Assay (UNLIM) Method" by D.E. Lenz is in press for publication in "Immunochemical Methods" Vol. B of "Methods in Enzymology".

PRESENTATIONS

Effects of Anticholinesterase and Anticholinergics on Triphosphoinostide Metabolism, presented at University of California, San Francisco, CA, March 1980 (by invitation). D. Maxwell.

Spin-Label Studies of Acetylcholine Receptor-Bearing Membranes, presented at the American Chemical Society Meeting, Las Vegas, Nevada, August 1980. C.A. Broomfield and L.B. McDonough.

In Vivo Target of Soman in Mammals, presented to Brigadier General Garrison Rapmund, Commander of US Army Medical Research and Development Command, at US Army Biomedical Laboratory, May 1980. D. Maxwell.

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TASK AREA ED/WORK UNIT 383

NEUROTRANSMITTER SYSTEMS INTERACTION: EFFECTS OF ANTICHOLINESTERASES AND TREATMENT COMPOUNDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG 6518	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 04 09	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY		3M161102BS10	ED	383
b. CONTRIBUTING	61102A			
c. CONTRIBUTING	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code) (U) Neurotransmitter Systems Interaction: Effects of Anticholinesterase and Treatment Compounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
012600 Pharmacology; 01680 Toxicology; 0023 Biochemistry; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04	CONT	DA	C. In-House

17. CONTRACT GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING		
	CURRENT		

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Biomedical Laboratory
ADDRESS: Aberdeen Proving Ground, MD 21010

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Shih, T. M.
TELEPHONE: 301-671-2373
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC:DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Neurotransmitters, (U) Organophosphorus Anticholinesterases, (U) P/T Compounds, (U) Cholinesterase, (U) Brain, (U) Gas Chromatography/mass Spectrometry, (U) Metabolism

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To elucidate the mechanisms by which chemical warfare agents affect, and explain the mechanisms by which drugs and other biological variables block the effects of chemical warfare agents on neurotransmitters.

24. (U) Employ recently developed sensitive and specific gas chromatographic/mass spectrometric method to quantify central putative neurotransmitter content at synaptic levels in discrete brain regions and in blood. Simultaneously, blood and brain area cholinesterase (ChE) activities will be analyzed.

25. (U) 8004-8009 Preliminary study of the effects of Soman and cholinesterase neurotransmitter acetylcholine (ACh), and its precursor, choline (Ch), showed that (1) acute subcutaneous injection of one LD50 of Soman (120 ug/kg) caused a differential degree of increase of ACh and Ch levels in different rat brain areas, with cerebral cortex having the highest elevation after 40 minutes; (2) chronic dosing of one-half LD50 of Soman (60 ug/kg, subcutaneously) once a week for up to 6 weeks did not produce any change in ACh and Ch contents in any brain areas investigated, but induced a moderate 25-40 percent) depression of cholinesterase (ChE) in brain stem, midbrain and cerebral cortex at 6 weeks.

*Available to contractors upon originator's approval

DD FORM 1498, 1 MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10 Research on Military Disease, Injury and Health Hazards

TASK AREA ES

WORK UNIT 383 Neurotransmitter Systems Interaction: Effects of Anticholinesterases and Treatment Compounds

INVESTIGATOR Tony Shih, Ph.D.

BACKGROUND

With the exception of a group of bicyclic phosphorus esters, most of the organophosphorus anticholinesterases, including powerful chemical warfare (CW) nerve agents, exert their toxic lethal effects by inhibiting the cholinesterase (ChE) family of enzymes, to include acetylcholinesterase (AChE). Presumably, this leads to accumulation of acetylcholine (ACh) at central and peripheral synaptic sites which then causesthe hyperactivity of cholinergic function. The most dangerous of these toxic actions appears to be the failure of respiratory and cardiovascular centers.

It is generally assumed that ACh will be elevated everywhere in the organism following exposure to any organophosphorus anti-ChEs. During the course of the literature search, however, only a handful of reports demonstrating the elevation of ACh in the brains of CW nerve agent-poisoned animals were to be found. A statement made by Holmstedt and his associates in their 1967 article seems appropriate here, "There are relatively few reports on the effects of ChE inhibitors on the ACh content in tissue and blood." To date, still there are few.

There are good reasons for this lack of information on ACh: first of all, CW agents are not available commercially; secondly, sensitive analytical procedures for ACh, and the introduction of microwave inactivation technology were only developed quite recently; thirdly, those investigators who have access to these nerve agents and work on ACh, concerned themselves mostly with protection of prophylactic and/or treatment drugs against the ACh elevation in the brain. Furthermore, those data that are available are for experiments performed with one dose and one time point, using different routes of administration, either intramuscular, subcutaneous, intravenous, or intraperitoneal. The data are difficult to consolidate and evaluate. Additionally, they are studies of activity changes in the whole brain.

The brain is a heterogeneous tissue both anatomically and histologically. This heterogeneity is of great importance in the evaluation and interpretation of biochemical findings related to behavioral manifestations. There are some specific neuronal tracts which travel from one region of nuclei to the other region of the brain. Additionally, each region which controls some specific function or behavior, has a varied amount of neurotransmitter contents. Furthermore, a balance between different neurotransmitter system activities exists in the central nervous system to control normal brain functions. Undoubtedly, a perturbation of one neurotransmitter system, such as the alteration of cholinergic activity by organophosphorus anti-ChE agents, will alter this balance. A number of articles have been published which demonstrate such notions and point to a belief that during organophosphorus anti-ChE poisoning, the interference of organophosphate with ACh metabolism was followed by a disturbance in the metabolism of catecholamines and other neurotransmitter substances, and these changes might be of pathophysiological significance.

These reported studies are, again, a one-shot investigation. There is either one dose or one time-period, and we do not have information on their dynamic time-course and dose-related effects. It is generally felt among neuroscientists that neurochemical studies on discrete brain regions will be more revealing in the sense of correlating neurochemical changes to specific behavioral patterns.

PROGRESS

During the period between April and October 1980, we have performed studies on two fronts: actue and chronic or repetitive exposures, respectively, of Soman administration on acetylcholine and choline levels in rat brain regions. Quantitative analysis of acetylcholine and choline in brain tissues was performed by a recently developed sensitive and specific gas chromatographic/mass spectrometric/ data system as described by Jenden et al (1973), after rats have been microwaved, focused on their heads and brain areas dissected-out.

Acute study.

We have investigated the time-course effects of a single subcutaneous administration of 9/10 LD_{50} of Soman (GD) on levels of acetylcholine (ACh) and choline (Ch) in six major rat brain areas, i.e., brain stem (including medulla oblongata and pons), cerebral cortex, hippocampus, midbrain, striatum, and cerebellum. As a comparison, identical experiments were also performed with diisopropyl fluorophosphate (DFP). So far we have followed 5, 10, 15, 20, and 40 minutes after injection of either agent. Preliminary data indicate that maximal elevation of acetylcholine is reached at 20 minutes and remains elevated at 40 minutes for both compounds at 9/10 LD_{50} dosage. As shown on Table 1, different regions of the brain have been affected differently and they vary with the different agents studied. Soman has more noticeable effects on cerebral cortex and hippocampus, whereas DFP produces more remarkable changes in brain stem, cerebral cortex and striatum.

Chronic study.

We have also examined the time-course effects of repeated administration of sublethal doses in rats of Soman (60-65 ug/kg, S.c.). Levels of ACh, Ch and acetylcholinesterase (AChE) were examined in six areas stated previously. Chronic dosing of 1/2 LD_{50} Soman once a week for up to six weeks induced a moderate (25-40%) depression of AChE in brain stem, midbrain, and cerebral cortex (cerebrum) at 6 weeks (Figure 1), but did not produce any change in Ach and ch levels in any brain areas investigated (Table 2) seven days after last injection.

If the dosings were increased to 3 times a week for up to six weeks, AChE activity showed a severe inhibition (45-75%) in all brain areas (Figure 2). However, even under these conditions of severely depressed AChE activities, the levels of ACh and ch in the six brain areas examined were not altered, 24 hours after last injection, as compared with control during the six-week period (Table 3).

TABLE 1

Percent changes of acetylcholine levels at 40 minutes following 9/10 LD_{50} subcutaneous injection of either Soman or DFP in rat brain areas.

Brain Areas	% change from control Soman	DFP
Brain stem	+ 6.88%	+ 26.85%
Cerebral cortex	+178.35%	+110.55%
Hippocampus	+ 80.05%	+ 31.29%
Midbrain	+ 15.94%	+ 23.28%
Striatum	+ 12.90%	+ 61.32%
Cerebellum	+ 51.87%	+ 42.81%

TABLE 2

Levels of acetylcholine in various rat brain areas seven days after last soman administration, once a week, for specified weeks.

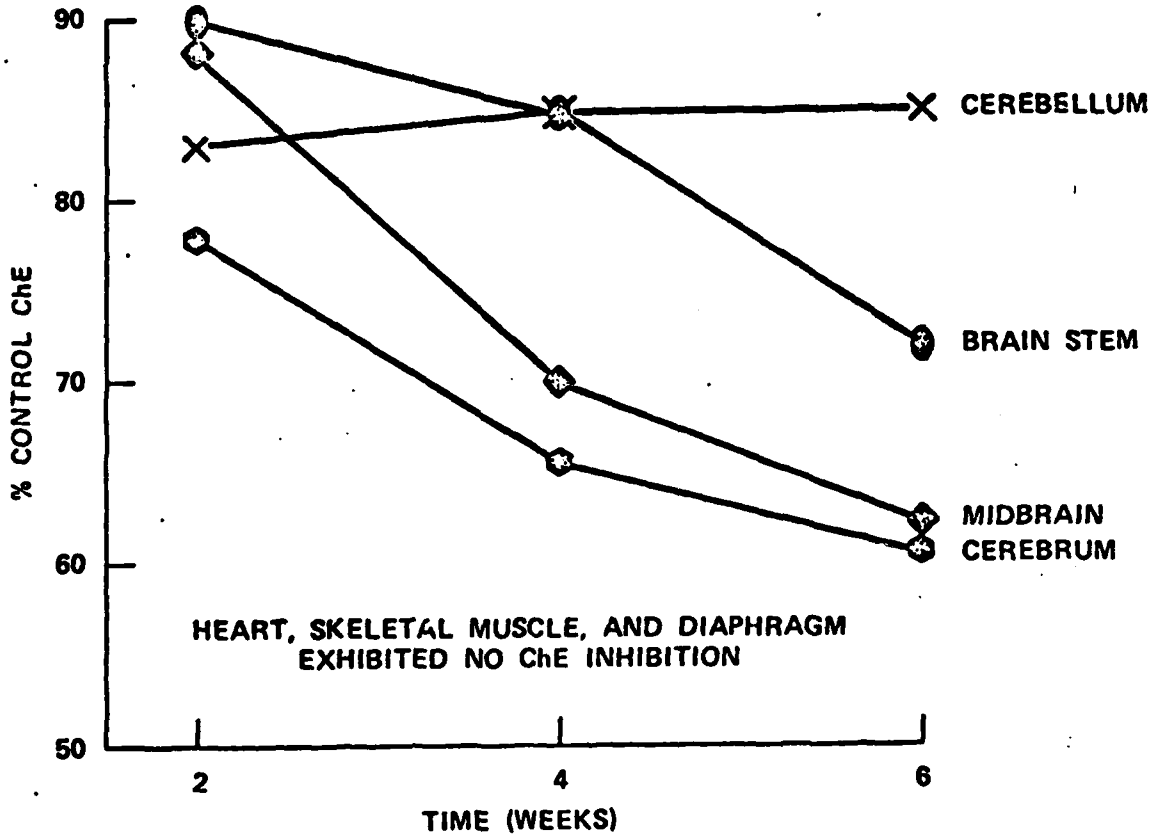
	Duration(wks)	Acetylcholine (nmoles/g) Control	Soman
Brain Stem	2	12.80 ± 1.28 (9)	9.01 ± 1.43 (9)
	4	13.36 ± 1.37 (4)	15.60 ± 4.38 (5)
	6	13.03 ± 2.81 (9)	13.06 ± 1.73 (8)
Cerebral Cortex	2	18.66 ± 1.51 (10)	18.96 ± 1.49 (9)
	4	18.58 ± 1.17 (5)	22.99 ± 2.24 (4)
	6	18.85 ± 1.43 (10)	16.78 ± 1.57 (9)
Hippocampus	2	17.66 ± 0.98 (9)	16.44 ± 2.24 (10)
	4	18.04 ± 4.52 (9)	17.90 ± 4.11 (8)
	6	17.42 ± 1.64 (9)	16.37 ± 2.90 (8)
Midbrain	2	21.28 ± 1.90 (10)	18.18 ± 2.19 (4)
	4	20.92 ± 0.60 (9)	21.69 ± 1.71 (6)
	6	22.06 ± 1.67 (8)	23.65 ± 1.89 (6)
Cerebellum	2	7.00 ± 0.58 (4)	4.97 ± 0.64 (5)
	4	5.96 ± 0.85 (4)	6.50 ± 1.33 (8)
	6	5.68 ± 0.48 (5)	6.45 ± 0.59 (4)
Striatum	2	60.22 ± 3.37 (5)	62.65 ± 6.42 (5)
	4	60.15 ± 6.36 (9)	66.15 ± 4.42 (8)
	6	59.91 ± 6.20 (5)	67.25 ± 9.99 (4)

Levels of acetylcholine in various rat brain areas twenty-four hours after last soman administration, three times a week, for specified weeks.

TABLE 3

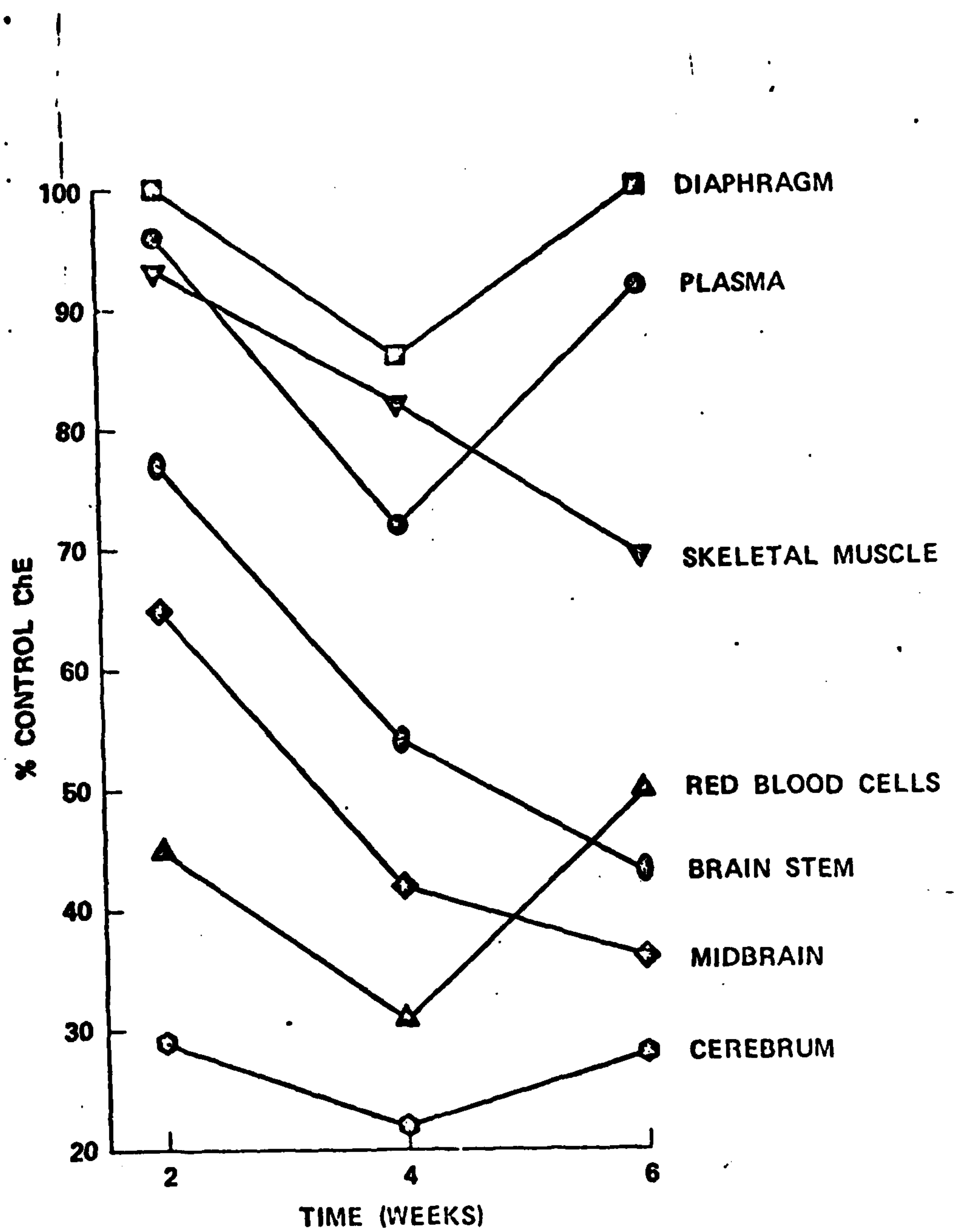
	Duration(wks)	Acetylcholine (nmoles/g) Control	Soman
Brain Stem	2	13.08 ± 1.61 (10)	14.10 ± 1.89 (8)
	4	12.28 ± 1.31 (10)	13.31 ± 1.56 (10)
	6	13.05 ± 1.58 (10)	9.86 ± 2.30 (5)
Cerebral Cortex	2	19.37 ± 1.40 (8)	23.83 ± 2.43 (8)
	4	20.10 ± 1.29 (7)	24.94 ± 1.87 (7)
	6	17.10 ± 1.34 (4)	21.36 ± 2.52 (4)
Hippocampus	2	15.05 ± 1.41 (8)	17.38 ± 1.22 (8)
	4	16.50 ± 1.16 (10)	18.06 ± 1.68 (10)
	6	15.84 ± 1.71 (8)	13.75 ± 1.90 (8)
Midbrain	2	21.17 ± 2.50 (10)	23.18 ± 2.09 (9)
	4	21.80 ± 1.76 (10)	21.04 ± 2.88 (9)
	6	21.02 ± 1.15 (5)	19.49 ± 0.73 (5)
Cerebellum	2	5.31 ± 0.51 (9)	5.87 ± 1.17 (9)
	4	6.03 ± 0.59 (10)	7.36 ± 0.74 (8)
	6	5.95 ± 0.70 (5)	5.29 ± 0.62 (5)
Striatum	2	63.07 ± 4.39 (9)	67.07 ± 6.43 (9)
	4	60.01 ± 4.98 (8)	52.81 ± 4.50 (10)
	6	60.27 ± 6.70 (5)	52.38 ± 6.68 (2)

Figure 1



Changes of cholinesterase activities seven days following last soman administration, once a week, for specified weeks in different rat tissues.

Figure 2



Change of cholinesterase activities twenty-four hours following last soman administration, three times a week, for specified weeks in different rat tissues.

PUBLICATIONS

Gas Chromatography, Mass Spectrometry and Combined Gas Chromatography-Mass Spectrometry, Hanin, I. & Shih, T.M., published in Physico-Chemical Methodology in Psychiatric Research (Hanin/Koslow), 1980.

PRESENTATIONS

An abstract entitled "Effects of Chronic Administration of Soman on Acetylcholine Metabolism," Shih, T.M., Lenz, D.E., Maxwell, D.M., accepted for presentation at Society for Neuroscience Annual Meeting -1-14 November 1980, Cincinnati, OH.

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